Fusarium wilt of oil palm: studies on resistance and pathogenicity

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FUSARIUM WILT OF OIL PALM : STUDIES ON RESISTANCE AND PATHOGENICITY

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To.

God the Almighty

creator of the universe be the Glory, for He has provided plants for man to use, so that he can grow his crops and produce bread to give him strength, wine to make him happy and oil to make him cheerful.

Psalm 104

FOREWORD.

Three papers (listed below) arising from this work have been accepted for publication and include results from sections 3 and 4 of this thesis. Reprints of the papers already published are included at the back of this thesis.

Flood, J., Mepsted, R., Velez, A., Paul, T .C. and Cooper, R .M. (1993). Comparison of virulence of isolates of *Fusarium oxysporum* f .sp. *elaeidis* from Africa and South America. *Plant Pathology*, **42** : 168 - 171.

Mepsted, R., Flood, J., Paul, T .C. and Cooper, R .M. (1994). Virulence and aggressiveness in *Fusarium oxysporum* f. sp. *elaeidis*; implications for screening for disease resistance. *Oleagineux*, **49** : 209 - 212

Mepsted, R., Flood, J., Paul, T. C., Airede, C. and Cooper, R. M. (1995). A model system for rapid selection of resistance and for investigation of resistance mechanisms in Fusarium wilt of oil palm. *Plant Pathology*, **44:** 749 - 755.

CONTENTS

ABBBEVIATIONS

ca	approximately.
CWDE	cell wall degrading enzymes.
d	day(s).
F.o.e.	*Fusarium oxysporum* f. sp. *eleaidis.*
F.o.a.	*Fusarium oxysporum* f. sp. *albedinis.*
g	gram.
h	hour(s).
M	moles or molar.
min	minute(s).
NS	Nelson Somogyi.
nm	nanometer (wave length unit).
PAL	phenylalanineammonia-lyase.
PG	polygalacturonase.
PL	pectin lyase.
PME	polymethylesterase.
PO	peroxidase.
PPO	polyphenoloxidase.
PVP	polyvinylpolypyrrolidone.
mRNA	messenger RNA.
RFLP(s)	restriction fragment length polymorphism(s).
R_f	reference value of a certain substance on tlc plates (in relation to solvent front)

SDW	sterile distilled water.
TLC	thin layer chromatography.
TWA	tap water agar (low nutrient medium).
VCG	vegetative compatibility group.
WR	wilt resistant oil palm line.
WS	wilt susceptible oil palm line.
WST	wilt standard oil palm line.

ABSTRACT

Fusarium wilt of oil palm is an increasingly serious problem in West Africa and the disease has recently been identified in South America. This is the first investigation to screen for resistance with several representative isolates from all oil palm growing regions and to develop a 'model' system which allows rapid screening of oil palm progenies and the study of mechanisms of resistance and pathogenicity involved in this host-pathogen interaction.

Variations in pathogenicity and aggressiveness among isolates world wide were demonstrated. Some highly aggressive isolates were identified from Africa and Brazil; an isolate from Ivory Coast was the most aggressive of all isolates tested. Pectic enzyme production both *in vitro* and *in vivo* was related to the degree of aggressiveness of the isolates. Some evidence indicated a possible genetic interaction between some Zairean isolates and seedlings. The pathogenicity of oil palm and date palm isolates was also compared and their ability to cross infect was apparent in this preliminary test.

An attempt to use an *in vitro* approach for rapid screening of lines for resistance using callus was not successful. Despite manipulation of hormones in the media and various inoculation techniques, resistance was not expressed at the callus stage. However, resistance or susceptibility were expressed in oil palm petioles and roots following infusion with conidia. Gels and tyloses were produced earlier, gels accumulated to higher levels and fungal colonization was restricted in resistant genotypes whilst in susceptible genotypes, production of gels and tyloses was delayed and the fungus

rapidly colonized the host tissue. Petioles from resistant clones (WR) remained green whilst those from susceptible (WS) clones became brown and there was good correlation with the conventional pathogenicity tests. Consequently, infused petioles can be used as a rapid screen (8 days *cf.* 8 months for conventional tests) for resistance. Also, infused petioles were used as a facile system to study mechanisms of resistance.

Antifungal compound(s) both preformed and induced were extracted from petiole xylem fluid and tissue in resistant palms only. These compounds accumulated such that 8 d after infusion with the conidia, tissue extracts completely inhibited spore germination in bioassays. However, identification of these antifungal substances remains unresolved.

A comparative study using infused petioles as a model system revealed activities of chitinase, glucanase and peroxidase but they were similar in both a resistant and a susceptible clone; activity of polyphenol oxidase was not detected.

The use of the infused petiole technique to study further the host-pathogen interaction and for practical use in identifying resistance in the field is discussed.

1.0 GENERAL INTRODUCTION

The Oil Palm (*Elaeis guineensis* Jacq.) is a large monoecious feather-palm. It has a solitary columnar stem with short internodes (Hartley, 1988), and is widely cultivated throughout the tropical regions of the world. Oil palm is thought to have originated from the forests of West Africa where it most frequently occurs as semi-wild groves, in areas where forest has been cleared. There are fossil, historical legend and linguistic evidences for its African origin (Hartley, 1988).

Oil palm has the potential for producing more vegetable oil per unit area than any other oil producing plant. Oil palm under good management and favourable climate, can produce up to six tones of oil per hectare per year (Jones, 1983). Oil (extracted from fruit kernel and pulp) accounts for approximately 15% of the world's vegetable oil and significantly contributes to the national economies of South East Asia, notably Malaysia, Indonesia, West African countries and tropical Latin America (Thurston, 1984).

Small quantities of palm oil have been imported into Europe mainly from West Africa since the 16 th century but the trade increased following the industrial evolution. The oil has been used as a raw material for the production of soaps, candles, margarines, cooking and recently, medically, in preventing river-blindness. In this latter case, supply was from native exploitation of semi-wild groves. Increasing demand for oil palm products has ,in recent years, stimulated the construction of commercial mills and the establishment of plantations in the Far East and in Africa. Currently, the oil palm is an established plantation crop throughout the equatorial regions of the world including South East Asia, which now supplies most of the world demand for palm oil (Hartley,

1988). However, while oil palm may be regarded worldwide as a commercial plantation crop, this is not the case in many parts of Africa. In Nigeria, for example, oil palm is highly regarded in the national diet. Sixty per cent of production comes from exploitation of semi-wild groves (Anon, 1991). In fact, to many millions of native Africans the whole plant can be used in a variety of way; palm wine is extracted from its fibrous stem or male inflorescence and is rich in vitamin B, the leaves of oil palm are used for thatching of houses whilst the petioles and rachis are used for fencing. In addition, petioles, rachis and bunches (after fruit has been removed) are burned to ashes and the ash which is rich in potash is used for traditional extraction of salt and in soap making.

Hartley (1988) suggested that such intensive and extensive native exploitation of the crop coupled with poor agronomic practices resulted in low yield of semi-wild palms. However, high yielding tenera seed varieties have been developed and are now available to small-holders in several African countries (Hartley, 1988; Anon, 1991; Kullaya, 1991).

There is no natural form of vegetative propagation in oil palm and thus it has not been possible in the past to multiply selected palms to produce high yielding clones. However, with the development of tissue culture techniques for oil palm, this problem has been overcome and will allow significant yield improvements.

While production in South East Asia has been on the increase in the past thirty years, exports from West African countries have been on decline partly due to civil disorder and increased internal demand (Purseglove, 1985; Hartley, 1988) but production in Africa has also been constrained by diseases, of which the most serious is considered

to be vascular wilt caused by *Fusarium oxysporum* f. sp. *elaeidis* (Renard, 1976; Turner, 1981).

Generally, vascular diseases are highly destructive, rapid in their effects and caused by fungal and bacterial pathogens. Wilt pathogens generally enter the vascular system of the host through the root system often said to be aided by entry through damaged tissue (Isaac, 1992). Further penetration occurs possibly after the production of pectic enzymes which attack the middle lamella between plant cells. The enzymes assist the fungus to invade living tissues. The majority of fungal wilt diseases are caused by species of *Fusarium* or *Verticillium.*

Fusarium oxysporum is a soil-borne pathogen with a worldwide distribution. Some strains are plant pathogens and some of these pathogenic forms are only poorly specialized and cause seedling blights, necrosis or rots. However, the forms responsible for vascular wilts exhibit a high degree of host specificity and they have been divided on this basis into more than 80 *formae speciales* (ff. spp.) (Louvet, 1988). Some ff. spp. have been further divided into races where they exhibit cultivar specificity within a host species.

Oil palm vascular wilt was probably first observed in Nigeria in 1944 and was described as "Lemon Frond" but the disease was considered to be a physiological disorder (Turner, 1981). In 1946, Wardlaw described a new vascular disease in Zaire (Belgian Congo) and *Fusarium* was isolated from diseased plants (Wardlaw, 1946 b and c). The disease was later recognized and confirmed in Nigeria in 1948 (Wardlaw, 1948) and (Fraselle, 1951) demonstrated the pathogenicity of *F. oxysporum* by inoculating the fungus into oil palm seedlings in a nursery. With the rapid expansion of

the oil palm industry, the disease was subsequently reported in many West African countries including Cameroon (Anon, 1960), Ivory Coast and Dahomey (Renard *et al.*, 1972) and widespread losses have occurred (Aderungboye, 1981). The disease has also been reported from Denpasa Estate, Brazil in 1983 (Van de Lande, 1983) and from Ecuador in 1986 (Renard and de Franqueville, 1989). The pathogenicity of a Brazilian isolate to clonal oil palm has been established (Flood *et al.*, 1993). There have also been unconfirmed reports from Surinam (Anon, 1951) and Colombia (Sanchez Potes, 1966).

The origin of the outbreaks in South America is unknown although seed-borne transmission is likely; the pathogen can be present on seed coats and on kernel surfaces with seeds (Flood *et al.*, 1992b). Also, isolates from Ivory Coast, Brazil and Ecuador are vegetatively compatible which may indicate a common origin (Flood *et al.*, 1992a). Contaminated seed can give rise to infected plants albeit at a low frequency and thus, an effective method of decontamination for seed from West Africa is required (Flood *et al.*, 1994). The disease has never been reported in Malaysia or Papua New Guinea. Without such a limiting factor, production has increased so that Malaysia is now the world's leading exporter of palm oil. Colhoun (1981) suggested that climatic differences, notably the lack of prolonged dry season in Malaysia could be a significant factor in disease expression. However, Ho *et al.* (1985a) considered that isolates of *F.oxysporum* from Malaysia were nonpathogenic to oil palm seedlings even under conditions of water stress.

Oil palm is vulnerable to *Fusarium* attack at all ages from seedling to mature palm and the disease can exist in two forms in the latter (Prendergast, 1957). Commonly, in the chronic form, the older leaves become desiccated and the rachises break near or at

some distance from the base hanging down around the trunk. The disease progresses gradually, with younger leaves becoming successively affected whilst the erect young leaves in the crown are much reduced in size and may become chlorotic; the palm can exist in this condition for several years. In the less frequent acute form of the disease, the leaves dry out and die rapidly while retaining their original erect positions on the plant until broken off, usually several feet from the base, by wind action. The disease progresses rapidly and palms die within two or three months. The stipe generally breaks off just below the crown and falls to the ground. Various intermediate stages between the acute and chronic forms may occur. Recently, de Franqueville and Renard (1990) have suggested a third category of temporary wilt where palms develop leaf symptoms but later recover. At the nursery stage, infected palms may show progressive shortening of younger leaves followed by desiccation and death of older leaves (Prendergast, 1957).

Internal symptoms of this disease are characterized by vascular discoloration from their normal pale yellow to brown and blockage of xylem vessels with tyloses, gels or gums (Kovachich, 1948; Ho, Vargese and Taylor, 1985b). Vascular discoloration is commonly observed in palm stems but in severely infected plants it can spread to the petioles (Turner, 1981).

In oil palm, the pathogen mostly enters the host through the roots and Renard (1970) suggested that the fungus was unable to penetrate undamaged roots. This was in contrast to Fraselle (1951) and Locke and Colhoun (1977) who demonstrated that the pathogen could penetrate through roots that had not been deliberately damaged in these experiments; inoculations were done at least one month after planting to ensure minimum root damage. Recently, Flood *et al.* (1989) also demonstrated that it was

possible to infect seedlings whose roots had not been damaged or disturbed. However, the primary entry site through the roots may be the pneumathodes, which are short modified roots in which the exodermis has ruptured exposing the cortical tissue and stele (Locke and Colhoum, 1977).

Losses of up to 50% have been recorded for palms under 10 years old in some plantations (Wardlow, 1950; Waterson, 1953; Guldentops, 1962; Renard and Quillec, 1983). However, in general, losses are low and have been estimated to range between one and two per cent per annum (Bachy, 1970; de Franqueville and Renard, 1990), and in some areas of West Africa wilt has never been observed in groves or plantations (Waterson, 1953; Aderungboye, 1981). Prendergast (1957) suggested that no yield reduction could be expected until more than 20% of palms had died, due to the increased vigour of adjacent palms. However, this yield adjustment could only be expected if diseased palms died quickly and the infected tolerant palms may eventually cause an overall yield reduction. When Renard and de Franqueville (1989) studied a population of palms in the field, they observed a 6% to 16% yield reduction in six year old palms where only 2.5% to 5.5% of plants had external symptoms. They attributed most of the yield reduction to the 20% to 30% plants that appeared to be healthy and yet were infected, with apparently no obvious wilt symptoms.

Environmental factors have been suggested to influence disease incidence for example where higher levels of wilt were observed in areas of low rain fall (Prendergast, 1957; Aderungboye, 1981). Prendergast (1957) further observed that drought induced root death made palms more susceptible to wilt disease and Waterson (1953) had previously observed higher levels of disease incidence at the end of rainy season. Cultural practices have also been reported to affect incidence of wilt disease.

Prendergast (1957) and Renard and Quillec (1983) observed higher levels of wilt in fields where oil palm has been replanted on sites of previously infected palms. Some practices such as the application of potassium (Prendergast, 1957; Renard and Quillec, 1983; Renard and de Franqueville, 1989) and removal of ground cover plants (Renard and Quillec, 1983) reduced disease incidence. However, Renard and de Franqueville (1989) observed that sterile used bunch stalks applied to palm bases after processing, increased diseased levels.

Despite efforts to find a fungicide, either systemic or otherwise, which would effectively control this disease, these attempts have proved to be both unsuccessful and uneconomical (Franselle, 1951; Guldentops, 1962; Moreau, 1960; Renard, 1973). Thus, most research for the control of this disease has been focused on breeding for resistance or tolerance. This subject and possible improvements to screening for and selection of resistant palm lines are discussed more fully in Section 3.

Studies on *Fusarium* wilt of oil palm have been in progress for more than forty years but there are still several important aspects of this disease that have either never been investigated or remain unclear. The inheritance of wilt resistance or tolerance is still not fully understood which is due partly to a lack of rapid test for resistance for both young palms in the nursery (symptoms take four to eight months to develop) and the difficulty of working with a tropical crop with a long breeding cycle in the field. Also, there is a lack of understanding of the underlying resistance mechanisms which could lead to more logical and facile screening methods. However, resistance mechanisms are difficult to study in this host pathogen interaction because of the chronic, asynchronous nature of this disease and because the morphology of young palms offer few tissues to test as 'model systems'. The swollen stem base (pseudobulb) has a

highly complex vascular architecture and problems of experimental replication arise with roots; removal of several roots from each plant can lead to plant death. A technique which exploits the ability of petioles or roots of oil palm to express disease resistance or susceptibility, thus providing a model system for rapid detection of disease resistant lines and for studying resistance mechanisms in this host pathogen interaction is discussed in more detail in Section 4.

2.0 MATERIALS AND METHODS

2.1 Plant Material and Conditions for Growth.

Oil palm seeds (from Plantation Lever au Zaire, Binga Zaire, Nigerian Institute For Oil Palm Research, NIFOR, Benin city) and seedlings from Plantation Experimentale R. Michaux, PB-Dabou, Cote-d'Ivoire (Table 1 a) were supplied either heat treated or requiring heat treatment to initiate germination (Hartley, 1988). Seeds requiring heat treatment were soaked in water for seven days at 25°C with daily changes of water, air-dried to a dull black colour, sealed in heavy duty plastic bags and incubated at 39°C for 80 days. Heated and preheated seeds (from Zaire and Nigeria) were again soaked in water at 25°C for seven days and air-dried as described above. Heated seeds were then sealed in airtight bags with an equal volume of air and incubated at 26°C to 27°C. Germination started between two to seven days and continued for two weeks. Seedlings were then transferred to plastic seed trays (300 x 220 x 50 mm) filled with Fisons F_2 compost: Fisons M_2 compost: Perlite in the ration 1:1:1. The seedlings were maintained in a controlled environment cabinet (at 28°C 80% R H and 12 h./ day with a light level of 240 μMol M^{-1} sec^{-1} photo flux density [P F D]). At 1-2 leaf stage, they were transferred into individual polyethylene bags (Plastics by Post, Isle of White) (80 x 190 mm) filled with 1.2L of compost before final transfer to the glasshouse. Ramets were supplied by Unifield T C Ltd., Cambridge Road, Bedford (Table 1 a). These ramets were initially placed in propagators to maintain a R H of 100%. The ramets were hardened off by gradually reducing the R H over a period of two months. The plantlets were then potted individually into black polyethylene bags (as above) and transferred to the glasshouse for inoculation.

To prevent splash-borne contamination between treatments in the glasshouse, seven or eight pots of the same treatment were placed in plastic troughs (610 x 160 x 140 mm, B-Line Swansea). In order to avoid overcrowding as the plants grew bigger, the number per trough was reduced to three or four. To further minimise any possibility of experimental irregularities in positioning plants in the glasshouse, plants within each treatment were frequently randomised between the troughs for that treatment. In all experiments, plants in the troughs were watered from below, as required, thus minimising any contamination. All plants were regularly fed at least once a month by watering from below with liquid fertiliser (Fisons Liquinure, 1 in 45 dilution, containing N.P.K. in the ratio 8:4:4 and trace elements). Initial spill pH at the beginning of the experiment was 5.0 and increased to 6.4 by six months. Although there were some variations in conditions in the glasshouse mainly due to the season these were always compensated for using shading and artificial lights (Complex 500w metal halide). Light levels were maintained between 800 and 500 u mol^{m-2} sec^{-1} with a day length of 14 to 17.; the ranges of humidity and temperature were respectively 60 to 90% and 22 to 35°C.

Table I a: Details of oil palm lines.

OIL PALM LINE.	ORIGIN
WS cross1	Zaire Joint Research Scheme Binga
WST cross	" "
WR cross 1	" "
WR cross 2	" "
Dumpy 100%	" "
Dumpy 75%	" "
Cross (a) seedling	Robert Micheaux Plantation Dabou Ivory Coast
Cross (b) "	" "
Cross (c) "	" "
Cross (d) "	" "
Cross (1)	NIFOR (Nigeria)
Cross (2)	"
WR clone 1/clone (UF28)	Uniliver (Unifield)
WS clone 1/clone (UF177)	" "
Clone UF136	" "
Clone A UF196 (WR)	" "
Clone B UF174 (WS)	" "
Clone C UF246 (WR)	" "
Clone D UF205 (WS)	" "
Clone E UF177 (WS)	" "
Clone F UF28 (WR)	" "
Clone 1 Coding confidential	" "
" 2 " "	" "
" 3 " "	" "
" 4 " "	" "
" 5 " "	" "
" 6 " "	" "
" 7 " "	" "
" 8 " "	" "
" 9 " "	" "
" 10 " "	" "
" 11 " "	" "
" 12 " "	" "
" 13 " "	" "

2.2 Fungal Isolates and Culture.

Twenty eight isolates obtained world-wide (Table 1 b) were used in the investigation. Pathogenicity and degrees of aggressiveness of these isolates were determined during these experiments. Stock cultures of all isolates were single spored and stored on sterile soil at 7°C to minimise growth and chance of loss of pathogenicity. Inoculum was obtained by plating a small quantity of soil onto potato dextrose agar (Oxoid, Basingstoke), and incubated for five days at 28°C. Five mycelial plugs (8 mm diameter) were removed from the actively growing edge of the cultures and placed into a 250 ml conical flask containing

150 ml of sucrose salts medium (Cooper and Wood, 1975), (Appendix 1). The cultures were maintained for five days in an orbital incubator at 100 rpm and 28°C. The fungal suspension was then filtered through two layers of muslin to remove mycelial aggregates. Concentrations of the resulting microconidia was determined with an haemocytometer and adjusted to 3×10^7 spores ml^{-1} unless otherwise stated with pH 6.5 sterile distilled water.

Table 1 b: Details of Isolates

ISOLATE	IDENTITY AND ORIGIN
F_3	*F. oxysporum* f. sp. *elaeidis;* diseased palm; Binga, Zaire
Y_1	*F. oxysporum* f. sp. *elaeidis,* diseased palm; Yaligimba, Zaire
BOS	*F. oxysporum*; soil; Bosonjo, Zaire
R_1	*F. oxysporum* var *redolens,* diseased palm; Yaligimba, Zaire
CAM	*F. oxysporum;* soil; Ndian Estate, Cameroon
LEY	*F. oxysporum;* soil; Layang-Layang, Malaysia
1379	*F. oxysporum* f. sp. *elaeidis* Diseased palm, Denpasa Brazil
Lobe 2	*F. oxysporum* f. sp. *elaeidis;* diseased palm; Lobe, Cameroon
Ndian3AR4	*F. oxysporum* f. sp. *elaeidis;* diseased palm; Ndian Estate, Cameroon
Ndian C_{10}	*F. oxysporum* f. sp. *elaeidis;* diseased palm; Ndian Estate, Cameroon
N1	*F. oxysporum* f. sp. *elaeidis;* diseased palm; Nigeria
OPC_4	*F. oxysporum* f. sp. *elaeidis;* diseased palm; Nigeria
OPC_1	*F. oxysporum* f. sp. *elaeidis;* infected root; Nigeria
Abak 508.1746	*F. oxysporum* f. sp. *elaeidis;* diseased palm; Nigeria
Abak 508.1322	*F. oxysporum* f.sp. *elaeidis;* diseased palm; Nigeria
16f	*F. oxysporum* f. sp. *elaeidis;* IRHO screening isolate; Ivory coast
146	*F. oxysporum* f. sp. *elaeidis;* diseased palm; Ivory Coast
G_4	*F. oxysporum;* soil; Benso plantation, Ghana
E_1	*F. oxysporum* f. sp. *elaeidis;* diseased palm; Ecuador
Cope 1	*F. oxysporum* f. sp. *elaeidis;* diseased palm; Cameroon
$F_2$4844	*F. oxysporum;* soil; Nigeria
Binga	*F. oxysporum* f. sp. *elaeidis;* diseased palm; Binga, Zaire
IC_3	*F. oxysporum;* soil, IRHO-Dabou, Ivory Coast
IC_5	*F. oxysporum;* soil; Palm Industry Tamobo, Ivory Coast
IC_9	*F. oxysporum;* soil; Ivory Coast
G1	*F. oxysporum;* soil; Benso Plantation, Ghana
C_2	*F. oxysporum;* soil; Cameroon
Albedinis	*F. oxysporum;* f. sp. *albedinis;* diseased date palm; Morocco

2.3 Inoculation of Plants.

Inoculation was performed by application of 10 ml of spore suspension with a plastic syringe, onto the soil surface around the base of each plant. To mimic the possible effect of culture nutrients on soil microflora, uninoculated plants (controls) received 10 ml of 10% sucrose salts medium. In these experiments, the plants were not disturbed nor was the root system deliberately wounded. Unless otherwise stated, seedling palms were inoculated at the 3 leaf stage whilst clonal plants were inoculated one month after transplanting and transferring to the glasshouse.

2.4 Inoculation of attached roots.

Roots still attached to 1-2 year old plants were inoculated with 0.5 ml *F.o.e.* conidia suspension (5 x 10^6 spores ml^{-1} unless otherwise stated). The roots were first separated and washed free of soil then severed under water at approximately 15 cm below the pseudobulb and transferred immediately to a suspension of spores for 3 h natural transpiration in the glasshouse. The outside of the roots was then washed, the first 1 mm removed from the cut end to reduce contamination and the root left *in situ* on the plant in the glasshouse until required. Control roots received sterile distilled water.

2.5 Inoculation of root segments.

Root segments(6 cm long) were cut with a razor blade below the pseudobulb. One end of each segment was dipped into 0.5 ml spore suspension as described above. Inoculum was drawn into the vascular tissue by applying a slightly reduced pressure to the other end of the segment by syringe or by rubber tubing attached to the water tap. Precautions were

taken to minimise damage to the root segments by using soft rubber tubing which also formed an effective seal with the root surface.

After inoculation the outside of the root segment was washed free of spores in sterile distilled water, blotted dry, the first 1 mm removed from the cut end to reduce contamination then placed in a humid chamber and incubated at 28°C until required. Control root segments were either infused with 0.5 ml sterile distilled water (wet control) or were incubated without infusion (dry control).

2.6 Inoculation of petioles with attached leaves.

Petioles from the youngest fully-opened leaves of 1 to 2 year old palms were severed under water (to prevent ingress of air) with a razor blade as described above, at approximately 20-25 cm from the top of the leaf. With leaves still attached, they were immediately transferred to an Eppendorf that contained 0.5 ml of 5 x 10^6 conidia ml^{-1} and allowed to transpire naturally for 3 h. in the glasshouse. Contamination was minimised as above. The petioles were then placed singly in flasks containing 100 ml of SDW (pH 6.5) and maintained in a humid chamber with a daily change of SDW until required. Control of petioles received SDW (pH 6.5).

2.7 Inoculation of petiole segments.

Petiole segments of the same age as above were inoculated as previously described for root segments (2.5) and controls were either infused with SDW (pH 6.5) or were incubated dry.

2.8. Assessment of Plant Growth and Disease Symptoms.

2.8.1 Plant height.

Plant height was measured three months post inoculation, and subsequently every 28 days. Measurements of the height were taken from soil level to the tip of the longest leaf. However, the position of the growing point in palms does not provide for plant height increase until the youngest expanding leaf has grown above the height of the previous leaf. Thus, despite adapting the measurement of the youngest fully expanded leaf as a better alternative it was not even accurate enough as an ideal indicator of overall plant growth. Consequently leaf chlorosis and necrosis were included in foliar disease assessments.

2.8.2 Leaf chlorosis and necrosis.

A chlorosis and necrosis index were used to express severity of disease. Plants were rated from 0 to 5, O = healthy, 1 = slight chlorosis of oldest leaves, 2 = significant chlorosis of <20% of leaves, 3 = significant chlorosis of <40% of leaves, 4 = significant chlorosis of >40% of leaves and 5 = plant dead).

2.8.3 External symptoms of petiole segments.

Following incubation, petioles were assess for external symptoms using a scale of 0 to 3 where, o = totally green, 0.5 - slight browning at one cut end, 1.0 = slight browning at both cut ends, 2.0 = browning more than 5 mm from cut end and 3.0 = section totally brown).

2.8.4 Vascular browning in pseudobulb.

At the end of experiments the plants were split longitudinally through the swollen stem base (pseudobulb) and the percentage area of the bulb stem with browning was assessed

using a stem area key (Plate 1a). Pseudobulb was defined as the central part of the bulb excluding leaf bases (Plate 1 b).

Plate 1 a: Stem tissue area key for assessment of stem browning in nursery palms

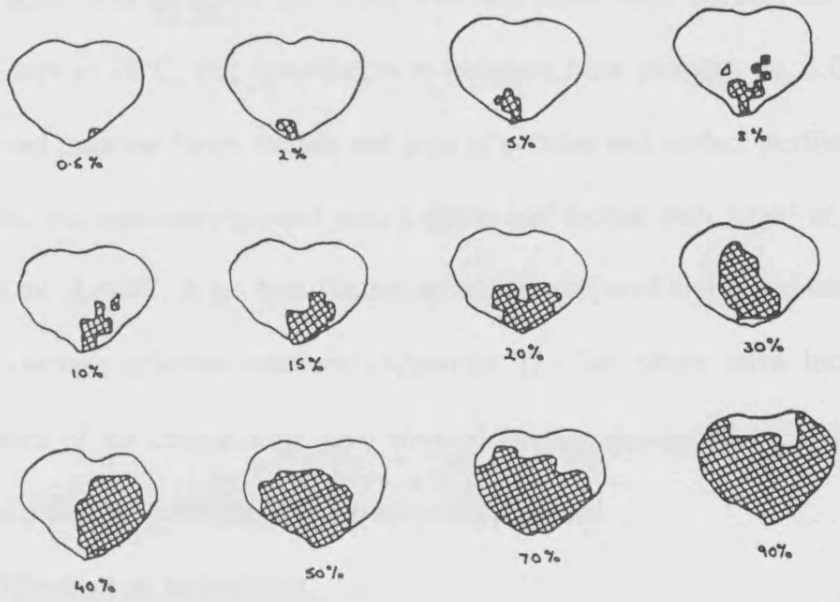


Plate 1 b: Longitudinal section through the bulb of an infected seedling, brown stem tissue indicates infection

2.8.5 Re-isolation and quantification of *F.o.e.* from plant material.

Qualitative re-isolation of the fungus on *Fusarium* selective medium (Papavizas, 1961) supplemented with antibiotics (Appendix 1) was also conducted. A 1 cm^3 block was removed from the bulb, surface sterilised in sodium hypochlorite (10% v/v) for 10 minutes and rinsed twice in SDW. The block was then plated onto the medium and incubated for four days at 28°C. For quantitative re-isolation from petioles, *ca.* 1.0 g. of tissue was removed from the bases, middle and tops of petioles and surface sterilised as above. Each section was separately ground with a pestle and mortar with 1 cm^3 of acid washed sand and 9 ml of SDW. A ten fold dilution series was prepared and plated onto duplicate plates of *Fusarium* selective medium (Appendix 1). The plates were incubated as above. Colonies of the fungus were then counted and the number of the colony forming units (cfus) per g. fresh weight of the tissue was calculated.

2.9. Histological techniques.

2.9.1 Light microscopy.

Sections were cut free hand, mainly of fixed (formalin acetic acid alcohol, FAA) tissue with a double edge razor blade. These sections were mounted directly into water and observed immediately. Lactophenol / cotton blue (0.1%) was routinely used as general stain for fungal hyphae in sections from infected material. These sections were also examined for vascular browning and occlusions (tyloses, gels). In germination tests, one drop of the stain was added to kill the spores prior to assessment.

The extent of vascular colonisation and occlusion was estimated by assessing the percentage of vessels containing hyphae, gels and tyloses. Three sections were made at

each point and mean values obtained. Observations were generally made at standardised intervals along each segment from the cut end or point of inoculation.

Micrographs were taken on an Olympus photosystem (PM-10 AD). Fluorescence microscopy was also performed on the same system with U.V. attachment, using exciter filter U and barrier filter Y475.

2.10 Measurement of vessel lengths.

Roots still attached to the plants or petioles with leaves attached were inoculated as above but inoculum suspension was mixed with fluorescent tracer particles. These particles were used to trace the initially infected vessels (Harrison and Beckman, 1982) and to identify primary trap sites which are presumably vessel end walls according to (Beckman *et al.*, 1962; 1976; Beckman and Keller, 1977). Freehand sections were cut as above, every 0.5 cm along each 10 cm long segment from the point of inoculation and the initial number of major xylem vessels at the cut end were counted. The sections were mounted in water and observed immediately by fluorescence microscopy. Vessel lengths were estimated by assessing percentage of vessels containing fluorescent particles.

2.11 Oil palm callus

2.11.1 Source and growth conditions.

Three lines of oil palm callus derived from WR and WS material were supplied by Unifield T.C. Ltd., and were maintained on basal agar (0.8%) medium (Murashige and Skoog, 1962). The cultures were subcultured every three weeks and one week before inoculation and were incubated at 25°C with a light regime of 16 h light and 8 h dark.

2.11.2 Inoculation of callus.

Callus cultures were inoculated by placing a 5µl drop of a conidial suspension ($1X10^6$ spores ml^{-1}) on a filter paper disc or disc of sterile cellophane which was placed on the surface of each callus piece. Alternatively, agar blocks (2 mm^3) cut from the actively growing edge of plate cultures of the fungus (on PDA), were placed on the surface of the callus pieces. Inoculated callus was incubated as above. controls received $2mm^3$ block of agar only.

2.11.3 Assessment of fungal colonisation on callus.

The extent of colonisation of the callus was observed daily and scored as follows: 0 = no fungal growth visible; 1 = aerial mycelia of fungus visible, but restricted to the point of inoculation; 2 = fungal growth nearly covering half of callus; 3 = fungal growth completely covering surface of callus; 4 = callus completely obscured and in most cases growing on surrounding medium.

2.12 Extraction of antifungal compounds.

2.12.1 Extraction from xylem fluid.

To remove antifungal compounds from petiole xylem fluids, a glass syringe fitted with solvent resistant tubing was used to flush diethyl ether through 5 cm long petiole segments. Ten µl of these xylem extracts forced through each petiole segment were collected into sterile cavity slides and evaporated to dryness.

2.12.2 Extractionfrom plant tissue.

Petiole or root segments each weighing 0.25 g from five replicate plants of each clone were roughly chopped with a clean razor blade and separately comminuted with 0.8 ml of

sterile sand and 8 ml of diethyl ether in a pestle and mortar. The homogenates were decanted, the filtrates evaporated to dryness in an air stream and redissolved in 2 ml of diethyl ether.

2.12.3 Extraction of tannins from plant tissue.

Five grams of petioles from five replicate plants within each cultivar were pooled and comminuted as above but in 25 ml of 2MHCl (BDH, Poole, Dorset, U.K.). The homogenates, in a pestle and mortar, were placed in a water bath and heated at 95°C for one hour and when solubilized, tannin components would appear red. After the colour change, 25 ml of n-butanol (BDH, Poole, Dorset, U.K.) were added and the contents again heated for one hour until the red colour phase separated and moved to the upper layer (Harborne, 1984). This phase was decanted into universal bottles and used in slide/chromatography bioassays for fungitoxicity. All extractions were conducted in low light to prevent possible photodegradation of compounds (Brinker and Seigler, 1993).

2.13. Bioassays for antifungal activity.

2.13.1 Spore germination.

Spore germination was assessed by slide bioassays technique adapted to evaluate the toxicity of crude plant extracts to spores of *F.o.e.* Cavity slides were immersed overnight in 75% alcohol, rinsed in SDW and dried in an oven at 85°C. Five day old *F.o.e.* liquid cultures were filtered through a double layer of muslin and centrifuged at 3000 g for five minutes to remove the culture fluids. Conidia were resuspended in SDW (pH 6.5) and diluted to 5×10^6 spores ml^{-1} . Unless otherwise stated, 50 µl from each test product (crude plant extracts in diethyl ether, solvent and water controls), were pipetted into each

cavity and allowed to evaporate to dryness before addition of 10µl of the conidial suspension. Crude plant extracts were dissolved in diethyl ether before addition to cavity and allowed to evaporate to dryness before addition of 10 µl of the conidia suspension. Each treatment was replicated five times. The slides were incubated over moist sterile filter paper in individual petri dishes at 25°C for 12h. After incubation, one drop of lactophenol/cotton blue stain was added to each slide to inhibit any further growth. Microscopic examination of germination and measurements of germ tube length were conducted at x200 magnification with graticule eye-piece. Conidia were considered to have germinated when the germ tube length was longer than the maximum diameter. Percentage of germination and mean germ tube length were calculated based on the germination of 100 spores and germ tube length of 20 spores, randomly quantified in five microscope fields from each cavity slide.

2.13.2 Thin-layer chromatography (tlc).

Thin-layer chromatography was used in attempts to separate and characterise antifungal compounds from crude plant extracts. Ascending tlc was carried out with precoated silica gel plates (60 tlc Merck 0.25 mm thickness with fluorescent indicator). Plates were washed before use by developing once in the solvent system to be used then preserved in aluminium foil. Developing tanks were presaturated with the solvent system to be used 2h before developing the plates at room temperature. Dried plates were viewed under U.V. light (254 nm) and R_f values calculated. Fifty µl of samples were applied 1.5 cm from one end of plates as spots 0.3 - 0.6 cm diameter with a micropipette. In order to obtain good

separation of the compounds in the crude extracts, the following solvent systems were used in succession (except system C); all mixtures are as v/v.

(a) Taquet *et al.* (1985): Hexane-Diethyl ether-methanol 5:5:1

Chloroform-diethyl ether-methanol 5:5:1

Diethyl ether-methanol-water 100:10:3 or 100:25:15

(b) Vernenghi *et al.* (1987): Hexane-diethyl ether 5:1

Hexane-diethyl ether-methanol 10:10:1

Chloroform-methanol 95:5

(c) Diethyl ether-methanol-petroleum ether 6:1:3

(d) Harborne (1984): Diethyl ether-petroleum ether 1:1

Ethyl acetate-benzene 9:11

(f) Two-dimension tlc on microcrystalline cellulose plates, Harborne (1984):

Benzene - acetic acid - water 6:7:3

Acetic acid - water 6:1

2.13.3 *Cladosporium* , Verticillium **and *Fusarium* bioassay (Karman and Sanfor, 1968).**

Plates were sprayed (Fisons Universal Aerosol spray) with a dense suspension of *Cladosporium herbarum* spores washed from 5d PDA plates with Czapek-Dox liquid medium (oxide) *Verticillium dahliae* or *F.o.e.* (5d liquid culture, filtered and diluted with FSM). After air-drying, the plates were incubated in a tlc tank in sterile, humid conditions and kept dark for 4d at 25°C. The plates were then examined for areas of inhibition of

growth of dark green *Cladosporium* mycelium, black growth of *Verticillium* mycelium, or white gowth of *Fusarium* mycelium.

2.13.4 Visualization reagents.

All compounds containing phenolic hydroxyl groups which have a free *para* or *ortho* position and which do not contain strongly de-activating substituents or sterically hindering groups react with diazotized amines at a suitable pH to give coloured azo dyestuffs (Dawson *et al.*, 1986).

(1) Diazotized p-nitroaniline.

a- A solution of the amine (5 ml, 0.5% w/v) in 2M - HCl was mixed with. sodium nitrite (0.5 Ml, 5% w/v), and sodium acetate (15 ml 20% w/v), was added.

b- $NaCO_3$ (20% w/v).

The two sprays (a and b) were used successively, the colour reaction with each being noted.

(2) Vanillin - sulphuric acid

This reagent was sprayed on tlc plates also for the detection of phenolics (Krebs *et al.*, 1969). The reagent consisted of 0.5 g of vanillin dissolved in 100 ml of a mixture of sulphuric acid-ethanol (4:1). Plates were sprayed and allowed to dry then heated in the oven to temperatures not exceeding 80°C for five minutes.

2.14. Extraction of enzymes from plant tissue.

Infected and control oil palm petioles were used for the extraction of enzymes. These petioles were frozen, chopped and finely comminuted in liquid nitrogen with a pre-cooled pestle and mortar. Enzyme extraction was performed in 0.025 M phosphate buffer (pH

6.0) containing 0.2M NaCl to dissolve enzyme from cell walls, 5mM dithiothreitol to prevent oxidation by phenoloxidases and 5 %w/v PVP to absorb phenolics (Cooper and Wood, 1980). Extraction was in the proportion 1 g tissue in 8 ml buffer, stirred for ten minutes. Large debris was removed by passing the mixture through a double layer of muslin then the filtrate was clarified by centrifugation (30 min./4°C/15, 000 x g) at 4°C. The supernatant was then dialysed overnight in distilled water and extracts maintained in ice at 4°C or stored at -70°C.

2.15.Enzyme assays.

2.15.1 Phenylalanine ammonia-lyase (PAL).

Activity of PAL in the supernatant was determined by measuring spectrophotometrically the production of cinnamic acid from L-phenylalanine (Bhattacharyya and Ward, 1988). The spectrophotometer was adjusted to null point at 290 nm. The reaction mixture, placed in a quartz cuvette, contained 2 ml of 50 mM, D or L - phenylalanine in phosphate buffer pH 8.One ml of enzyme extract was added to the mixture and OD at 290 nm read against water blank. The samples were incubated during 2 h at 35°C. PAL activity was expressed in nKats.

2.15.2 Peroxidase (PO).

Freshly prepared pyrogallol reagent was made by mixing 10ml 0.5 M pyrogallol solution and 12.5 ml of 0.66 M phosphate buffer (pH 6.0) and making the volume up to 100 ml with distilled water). Two ml of enzyme extract and 2.0 ml of pyrogallol reagent were thoroughly mixed in a cuvette. The increase in absorbancy at 240 nm, after the addition of 0.5 ml of 1% hydrogen peroxide was recorded at 25°C every 15 seconds for 3 minutes.

Enzyme activity is expressed as change in OD $min.^{-1}$ (90 - 30 sec.) at 420 nm (Lobenstein and Linsey, 1961).

2.15.3 Polyphenoloxidase (PPO)

The presence of PPO was investigated by adding 1 ml of enzyme extract to 1.5 ml phosphate buffer (0.1 M, pH 7.0) in a cuvette. At time zero, 1 ml of 0.4% catechol was added and thoroughly mixed. Activity was recorded as the increase in absorbancy at 495 nm and expressed as change in OD min^{-1} (90 - 30 sec.) ml^{-1} at 25°C (reagents and enzyme extracts were pre-incubated in a water bath before adding to the cuvette). Readings were continued for longer periods when activity was low. Controls were calculated from readings of enzyme solution minus catechol and catechol minus enzyme solution (Cooper, 1974).

2.15.4 Glucanase.

Glucanase activity was investigated by a modification of the method of Keen *et al,*. (1983). This activity was determined by measuring the release of reducing sugars (Reissing *et al.,* 1955) from assay mixture containing 250 µl laminarin (Sigma, at 200 mg/ml in 50 nM K acetate buffer pH 5.3) using Nelson Somogyi (NS) as test reagents. Time zero samples contained 1 ml NS reagent plus substrate and enzyme extract. After incubation (2 h at 35°C) 1 ml of NS reagent was then added to the rest of the samples, boiled for 15 mins, cooled for 2 mins and finally mixed thoroughly with 1 ml NS reagent 2 and left overnight . The insoluble substrate (undigested laminarin) was removed by centrifugation (10,000 g, 5 mins.) and the absorbancy measured at 620 nm against a

substrate blank. The increase in glucose was read from a standard curve (Appendix 5) and enzyme units are calculated as nKats.

2.15.5 Chitinase.

A modified method of Pegg and Young (1982) was used to investigate the presence of chitinase. The reaction mixture used was as above but colloidal chitin (from crab shells (Sigma at 2 mg/ml in 0.1 M sodium acetate buffer, pH 5.0) was substituted for laminarin. NS reagents were used to test for release of reducing sugars and enzyme activity calculated as above but using an n-acetylglucosamine standard curve (Appendix 5).

2.15.6 Pectic enzymes.

2.15.6.a Pectin lyase assay.

Pectin lyase (PL) activity was assayed spectophotometrically at 30°C by measuring the changes in absorbency at 250nm using a Shimadzu U.V. 260 spectrophotometer. Reaction mixtures contained 0.6 ml 0.25% w/v pectin in 0.05 M Tris-HCl buffer (pH 9.0), 0.3 ml enzyme extract and 0.1 ml $CaCl_3$ (0.01M final concentration in reaction mixture. Enzyme activity are expressed as units (µmol substrate released ml^{-1} h^{-1}) (Dow *et al.*, 1987).

2.15.6.b Endo-polygalacturonase assay.

Activity of endo-polygalacturonase (endo-PG) was determined by viscometric assay with 8 ml of 1% (w/v) sodium polypectate (Napp) in 0.1 M citrate buffer at pH 5.0 and 2 ml of enzyme extract in reaction mixtures. Activities are expressed as relative viscometric units

(RVU), defined as 10^3 x the reciprocal of time (min.) (t_{50}) for 50% decrease in relative viscosity of a reaction mixture. Technico 200 viscometers were used in a water filled tank at 25± 0.5°C (Cooper and Wood, 1975).

PG activity in the culture filtrates was also estimated by 'cup-plate' assay method which detects overall PG activity (exo and endo). The 'cup-plate' assay was performed as described by Dingle *et al.* (1953). The substrate, sodium polypectate (Sigma) was incorporated at 1% in 0.2 M phosphate buffer, pH 5.3 ammonium oxalate was included at 0.5% (w/v) to remove any calcium present and 0.01% salicylanilide added to prevent fungal growth. Agar was present at 15 g per litre. The molten mixture was poured into 9 cm diameter petri dishes (20 ml per dish) and allowed to set. Cups (7mm in diameter) were cut with a cork borer, 3 to a plate and 50µl duplicate samples of culture filtrates and heat inactivated enzyme (which served as a control) were added to these before incubating the plates at 30°C for 18 h. Zones of enzymatic activity were visualized by flooding the plates with 5N HCl. The zone diameter was measured (excluding the actual cup well size) and $\log_{10}$ of this diameter related to enzyme activity expressed as units pectinase. Pectinase from *Aspergillus niger* (Sigma) was used to construct a standard curve of $\log_{10}$ enzyme activity against zone diameter. Pectinase was used at concentrations between 0.00064 and 10 units per 50 µl where 1 unit will liberate1 µ mole of galacturonic acid from polygalacturonic acid per min. at pH 4.0 at 25°C.

2.15.6.c Pectin Methylesterase assay.

Pectin methyl esterase (PME) was assayed by an adaptation of the continuous titration method as described by Bateman (1963). The reaction mixture contained 4 ml of enzyme

with 10 ml of 1.5% citrus pectin in 0.15 M NaCl at pH 5.5. After 3 h incubation at 30°C the mixtures were titrated with 0.025 M NaOH required/min/ml filtrate. All assays contained heat inactivated enzyme as controls.

2.16 Statistical analysis.

Data were analysed by Minitab (Minitab Inc. USA) or Statsease (B. Clarke, University of Nottingham) statistical programmes. Normality of data was tested by the n-scores method or by observation of the data in the form of histograms. Homogeneity of variance was tested by Bartlett's test or by the F Max method (Parker, 1979). If the data were found suitable, an analysis of variance was performed, followed (as appropriate) by Bonferroni's inequality test (an unplanned comparison of means).If data were not suitable for analysis of variance, i.e. non-parametric, then a Kruskal-Wallis test was performed which, if significant, was followed by an STP non-parametric multiple comparisons tests (Sokal and Rohlf, 1981) for unplanned comparisons, or repeated Mann-Whitney U-test (Seigel, 1956) for planned comparisons. Examinations of data in the form of ratios were performed by Chi-squared analysis of contingency tables (Mead and Curnow, 1983) and, as appropriate, by Fisher's Exact test (Seigel, 1956).

3.0 SCREENING FOR AND SELECTION OF DISEASE RESISTANT OIL PALM LINES

3.1 INTRODUCTION.

Fusarium oxysporum f. sp. *elaeidis* is a soil-borne pathogen; its nature combined with the scale of oil palm plantations, the perennial nature of the crop and the relatively low value of the crop has made chemical control of this disease impracticable. Consequently most research has been directed towards the screening for and selection of disease resistant cultivars.

Wardlaw (1946; 1950) observed large differences in the incidence of wilt in adjacent blocks of field palms, and suggested that genetically controlled resistance may exist. He proposed that to save time and space, breeding programmes should be expanded to include screening for resistance using seedlings, which were also susceptible to the disease. These ideas formed the basis for the identification of resistant materials in trials in which plants were artificially inoculated with *F.o.e.* such methods are now used for the assessment of over 600 seedling crosses per annum in the Ivory Coast (de Franqueville and Renard, 1990).

Detailed studies and utilization of host resistance requires an understanding of the genetics of the host / pathogen interaction and the reliability of the nursery test depends on the use of a wide range of isolates that are representative of the areas where oil palm is grown. It is considered that although isolates of *F.o.e* may vary in the degree of symptoms they induce (aggressiveness), they do not significantly differ in their ranking for resistance of

different palm crosses (virulence) i.e. races of *F.o.e.* do not appear to exist. However, pathogenic races have been demonstrated in other *Fusarium* form species affecting tomatoes, (Gerdemann and Finley, 1951), banana (Buddenhagen, 1990) and peas (Goth and Webb, 1981). The existence of races of *F. oxysporum* f.sp *cubense* on banana is of particular interest here because like oil palm, banana is also a monocotyledonous plantation crop.

Lack of evidence for the existence of *F.o.e.* races has been highlighted in several investigations. Prendergast (1957) found no differential interaction in isolates obtained from diseased palms in and around Cowan estate in Nigeria, and although Obuekwe and Osagie (1989) reported differences in symptom development induced by isolates from Nigeria, Zaire and Cameroon, there appeared to be no isolate/cross interaction. Furthermore, no difference in virulence was found for isolates obtained from palms exhibiting the acute or chronic forms of wilt in the Ivory Coast (de Franqueville, 1991). The failure to observe differential interactions in nursery trials, led de Franqueville (1991) to conclude that the selection of resistant material in the Ivory Coast based on inoculation tests with one or a few isolates was valid.

Evidence contradicting these studies emerged when palms bred for resistance in the Cameroon and Ivory Coast were reported to be much more susceptible under field conditions in Nigeria than resistant material selected using Nigerian isolates (Aderungboye, 1981; Oritsejafor, 1989). In particular, the material selected as resistant in the Ivory Coast had the highest incidence of wilt (47%) anywhere in Nigeria under field conditions (Oritsejafor, 1989). Conversely, progenies selected for tolerance against

Nigerian isolates proved susceptible when planted in the field in Ivory Coast (Renard, 1991 pers.comm.), and material resistant to Zairean isolates was susceptible to an isolate from Brazil (Flood *et al.*,1993).

Field and nursery trials have also led to many theories on the nature of host resistance; some contradictions have emerged. For example, in a study on a wide range of palm crosses, Prendergast (1963) and Meunier *et al.* (1979) observed that some palms in every cross tested were infected in the nursery and no plants showed immunity and they concluded from their work that resistance was polygenic. However, Beckman (1987) reported that such trials involving severe inoculation of plants in the nursery, and any other wilt diseases where resistance is controlled by strong, single genes, a breakdown of immunity is highly likely to occur.

In contrast, Meunier *et al.*(1979) demonstrated strong evidence for polygenic control of resistance when they crossed 8 palm lines, ranking from highly susceptible to resistant and tested the progeny for resistance in nursery trial. They showed that there were both resistance and susceptibility genes and that these genes were present in high numbers and they suggested an additive effect.

The idea of polygenic resistance led workers at I.R.H.O. (Institut de Recherches pour les Huiles et Oleagineux) to suggest that within a cross, individual palms would have a similar number of resistance genes. They described the failure of some infected palms to exhibit symptoms as a reflection of the "threshold" nature of disease resistance, rather than segregation of resistance genes within a cross. They concluded that the relative resistance

of a cross could be estimated by the percentage of infected plants that became diseased (Meunier *et al.*, 1979).

In contrast to this the additive theory, detailed studies on the development of disease in the genealogical block in an oil palm plantation at Binga in Zaire, de Franqueville and de Greef (1987), observed a wide range of losses in different progenies (from 0 to 90%). Furthermore, in crosses between the most resistant and susceptible palms, the level of resistance did not appear to have been inherited in an additive manner; some form of segregation occurred. From these observations, they postulated an alternative theory for the inheritance of resistance i.e. that resistance was controlled by 2 genes, which conferred different types of resistance, segregated independently and had additive effect. For each gene, one allele governed resistance and the other susceptibility and thus a heterozygote had an intermediate level of resistance conferred though semi-dominance. This theory suggests that resistance genes would be unevenly distributed between palms within a cross and large differences in wilt resistance of clonal lines produced from trees of the same cross have been reported.

In support for the theory of simple genetic control of resistance, Renard *et al.* (1980) were able to show immunity to infection at the nursery stage with some lines of *Elaeis oleifera* (South American oil palm). However, since this immunity had never been observed in any *E. guineenisis* crosses, yet could be transmitted to some *guineenisis x oleifera* hybrids, they concluded that the resistance characteristic was different to those of *E. guineensis* and was due to a "simple genetic determinant".

Thus, the genetic mechanisms controlling disease resistance in oil palm remain unclear and much more work on the genetics of disease resistance is required before final conclusions can be drawn (de Franqueville, *et al.,* 1991).. Differences in methodology of trials at both nursery and field levels may partly explain the contradiction between the theories concerning polygenic and 2 gene inheritance respectively and the wider genetic base at Binga in Zaire upon which de Franqueville and de Greef (1987) based their hypotheses of hereditary transmission of resistance in oil palm.

Another palm species, date palm (*Phoenix dactylifera L.)* coexists with oil palm in some African countries including Nigeria, Burkina Faso and Sudan. The semi-arid climatical conditions in the northern parts of these countries favour growth of date palm for both local and commercial consumption while oil palm is grown in the Southern parts.

Fusarium wilt of date palm or "Bayoud" caused by the soil-borne fungus (*F. oxysporum* f. sp. *albedinis*), is the most important disease of date palms in the world (Killian and Maire 1930; Djerbi, 1982). The fungus can be spread by water, wind and can be transported (to other date palm growing areas) and preserved for a long period of time in soil, infected wood or pieces of rachis, offshoots (propagation material) and in craft items produced from date palm tissues, such as pack-saddles, baskets and ropes (Malencon, 1950). When such articles are discarded in a moist place favourable to the fungus it can resume growth, become established in the soil and infect palms (Pereau-Leroy, 1958). Although floral infection by this fungus has been shown Malecon (1941), the establishment of Bayoud in the palm as a result of floral infection has not been demonstrated.

The possibility of cross infection by the two ff. spp. adapted to palms is yet unknown and some attempts have been made in this study to investigate this possibility. There has been a report of the isolation of *F.o.a* from *Phoenix canariensis,* another species of palm. Similar symptoms were observed as with fusariose of date palm . (Djerbi, 1982).

An alternative approach to screening for resistance in whole plants is the use of *in vitro* methods. The use of tissue culture systems to screen for resistance and to study various aspects of host-pathogen interactions has certain advantages over studies utilizing intact plants. These include more rapid screening and thus more tests conducted because of space and time constraints being reduced. Also, the simplicity offered by the presence of one or a few plant cell types and the ability to control environment and nutritional factors are other advantages (Brettel and Ingram, 1979; Helgeson *et al.* 1972; Holliday and Klarman, 1979). Nonetheless, results from studies of this type must be usually interpreted with caution (Brettel and Ingram, 1979).

In addition *in vitro* techniques may be used to select for resistance. Carlson (1973) first demonstrated that plant cells and protoplasts could be selected in culture for resistance to methionine toxin and that plants with an altered response to infection by the pathogen could be regenerated from these cultured cells. Since then there have been many reports of *in vitro* selection for disease resistant germ plasm. However, resistance may not be expressed *in vitro*; tissue culture may present an unnatural environment because whilst separated, friable cells offer uniformity of infection they may not offer a collective resistance response. Also, the pathogen may overgrow the host cells and the culture medium perhaps saprophytically, thereby overwhelming potential defences through

unrealistically high inoculum level (Daub, 1986; Miller *et al.*, 1984 and Van den Bulk, 1991).

Expression of resistance in tissue culture systems may depend on several factors, including inoculum concentration, temperature and medium components such as phytohormones (Helgeson and Haberlach, 1980). One of the best ways to test whether resistance can be expressed at the cellular level is to compare the response of tissue cultures of resistant and susceptible varieties to pathogen inoculation (Daub, 1986). Thus, in experiments conducted on tobacco callus culture, Haberlach *et al.* (1978) and Deaton *et al.* (1982) were able to show resistant or susceptible reactions at tissue culture level but no differences could be shown between the response of resistant and susceptible lines in culture cells of sugar beet and tobacco respectively (Ingram and Joachim 1971; Reed and Rufty, 1985). However, using modifications of hormonal regimes in the growth medium, and a tissue culture line derived from resistant tobacco plant Helgeson *et al.* (1972) were able to vary colonization rates (to race 0 of *Phytophthora parasitica var. nicotianae)* over a wide range; from relatively slow to colonization rates much faster than in callus tissue from susceptible plants.

It should be noted that some success has been achieved with vascular wilt fungi including work conducted on callus cultures of lucerne challenged with *V. albo-atrum* (Latunde-Dada and Lucas 1985) and of tomato with *F. oxysporum* f. sp. *lycopersici* (Kroon *et al.*, 1991; Kroon and Elgersma, 1993).

Against this background, it was decided to explore the possibility of early screening for disease resistance in oil palm lines at tissue culture level.

The main objectives of this section are therefore:-

1. Screening crosses and clones for resistance at the nursery stage with *F.o.e.* isolates from all major oil palm growing areas in order to identify extremes of susceptibility and resistance for use in studies on resistance mechanisms (Section 4).

2. Investigation of the existance of a genetic interaction with oil palm material and *F.o.e* isolates.

3. Investigation of the expression of resistence *in vitro* with a view to producing a rapid screeen for resistance.

RESULTS.

3.2.1 Pathogenicity of seven isolates of *F.o.e.* on six oil palm lines of known wilt status.

In breeding programmes in Africa, selection for resistance at the nursery or prenursery stage involves artificial inoculation using one or more pathogenic isolates and sometimes mixed cultures of several isolates are used (Renard *et al.*, 1972; Obuekwe and Osagie, 1989). As planting materials are constantly being introduced to new areas, the resistance of the material when encountering local fungal isolates should be investigated. Thus, for the first time, an investigation of the comparative pathogenicities of a wide range of isolates from different areas under controlled environmental conditions was undertaken.

Six oil palm lines (4 crosses and 2 clones) of known wilt status were inoculated (2.3) with seven isolates. Final disease assessments were conducted as previously described (2.8), and these were made seven months post inoculation. Clones and crosses are referred to by their number and wilt status where WS = wilt susceptible, WR = wilt resistant and WSt = standard cross with no selection for resistance.

Soil isolates from Malaysia (LEY), Cameroon (CAM) and Zaire (BOS did not induce significant symptoms in any oil palm tested (Tables 2a - c). Even where there appeared to be some low levels of infection with the Zairean soil isolate, BOS, (WS cross 1) generally, like the Malaysian and Cameroon isolates, it failed to induce significant symptoms. This fungus, nonetheless, was able to penetrate WS and WR seedlings. Soil isolates were reisolated from the bulb tissue of inoculated WS clones or crosses but rarely from the

resistant lines with the exception of BOS (Table 2 d). It was further observed that these soil isolates rather than reducing growth appeared to slightly promote growth of some oil palm lines as compared to the controls although this was not significant statistically (Table 2 a).

Table 2a: Growth* of oil palm lines following inoculation.

	OIL PALM LINE					
ISOLATES	**WS CLONE 1**	**WR CLONE 2**	**WS CROSS 1**	**WST CROSS**	**WR CROSS 1**	**WR CROSS 2**
LEY		91.0 a	112.8 a		117.0 ab	117.3 a
CAM	94.4 a	113.7 a	104.4 a	114.3 ab	125.0 a	105.0 a
BOS		101.4 a	87.3 ab		105.9 ab	104.8 a
Y1	57.6 b	98.8 a	65.0 bc	105.9 ab	109.3 ab	79.1 b
R1	59.5 b	100.0 a	61.6 bc	93.6 b	105.9 ab	88.1 ab
F3	61.1 b	93.7 a	67.6 bc	112.3 ab	96.4 bc	86.4 b
1379	46.3 b	89.7 a	56.4 c	70.2 c	72.4 c	75.1 b
CONTROL	89.4 a	104.5 a	100.1 a	121.7 a	121.3 ab	103.4 ab

Values represent a mean of 16 replicates.

Within each column, values with the same letter are nsd ($p>0.05$) using Kruskal Wallis and repeated U-tests.

*Growth is the mean height (cm) of youngest fully expanded leaf measured at 28 day intervals from 3 - 7 months post inoculation.

The Zairean isolates, Y_1, R_1 and F_3 as well as the Brazilian isolate 1379 induced severe stunting (51 - 68%) (Table 2 a). These isolates also caused extensive vascular browning in the bulb tissue (Table 2 c) and the majority of inoculated palms contained the pathogen

(Table 2 d). Wilt susceptible clone 1 (UF 177) was therefore, confirmed as susceptible to these four isolates

Table 2 b: External symptoms exhibited by inoculated oil palm lines.

	OIL PALM LINE					
ISOLATES	**WS CLONE 1**	**WR CLONE 2**	**WS CROSS 1**	**WST CROSS**	**WR CROSS 1**	**WR CROSS 2**
LEY		0 a	0 a		0 a	0 a
CAM	0 a	0 a	0 a	0 a	0.1 a	0 a
BOS		0 a	0.4 a		0 a	0.3 a
Y1	0.6 a	0 a	1.4 ab	0.2 a	0.2 a	1.4 b
R1	0.6 a	0 a	2.0 b	0.9 b	0 a	0.8 ab
F3	0.9 ab	0 a	1.9 b	0.2 a	0.7 a	1.1 ab
1379	1.9 b	0 a	2.7 b	2.1 c	0.9 a	1.9 b
CONTROL	0 a	0 a	0 a	0 a	0 a	0 a

Values represent a mean of 16 replicates

Wilt index based on disease score 0 - no symptoms - 5 - plant death. (Flood *et al.* ,1989)*

*This observation was recorded at 28 day intervals from 3 - 7 months postinoculation.

When inoculated clones were compared it was observed that WR clone 2 did not show external wilt symptoms (Table 2 b) although some stunting was apparent with plants inoculated with isolate 1379 but even so this was not significant. Some slight vascular necrosis was also observed (Table 2 c) but again this was not significantly different from the controls. However, these isolates penetrated the clones except isolate Y_1 which was not reisolated from the tissue of WR clone 2 (Table 2 d).

Table 2c: Internal symptoms* exhibited by inoculated oil palm lines

	OIL PALM LINE					
ISOLATES	**WS CLONE 1**	**WR CLONE 2**	**WS CROSS 1**	**WST CROSS**	**WR CROSS 1**	**WR CROSS 2**
LEY		0 a	1.2 a		0 a	0 a
CAM	4.9a	0 a	10.5 a	0 a	0 a	0 a
BOS		1.3 a	34.2 ab		13.0 ab	9.5 ab
Y1	39.7 b	8.7 a	53.9 bc	3.1 a	5.9 a	53.2 c
R1	39.3 b	3.2 a	58.9 bc	19.7 a	5.9 a	28.1 abc
F3	42.8 b	14.7 a	63.1 bc	18.4 a	32.8 bc	36.7 bc
1379	67.9 b	12.3 a	78.4 c	50.8 b	43.7 c	56.2 c
CONTROL	0 a	0 a	0 a	0 a	0 a	0 a

Values represent a mean of 16 replicates

Within each column, values with the same letter are nsd ($p>0.05$) using Kruskal Wallis and repeated U tests.

*Internal symptoms were assessed 7 months post inoculation as % browning of stem tissue in the bulb (2.8.4).

When seedling crosses were inoculated with isolates Y_1, F_3 R_1 and 1379, it was observed that WS cross 1 showed severe stunting (56 - 67% reduction in height) (Table 2a); widespread leaf chlorosis and necrosis (Table 2 b) and significant vascular browning in the bulb (Table 2 c). Susceptibility of cross 1 was thus confirmed when these isolates were reisolated from the majority of inoculated palms (Table 2 d).

Table 2d: Reisolation* of *F. oxysporum* from inoculated oil palm lines.

	OIL PALM LINE					
ISOLATES	**WS CLONE 1**	**WR CLONE 2**	**WS CROSS 1**	**WST CROSS**	**WR CROSS 1**	**WR CROSS 2**
LEY		0:16 a	1:15 a		2:14 a	2:14 ab
CAM	8:8 a	1:15 ab	3:13 ab	1:15 a	1:15 a	0:16 a
BOS		0:16 a	9:7 bc		12:4 bc	6:10 bc
Y1	14:1 b	0:16 a	12:4 cd	5:11 ab	6:10 ab	13:3 de
R1	12:1 b	2:14 ab	12:4 cd	13:3 c	6:10 ab	8:8 bcd
F3	11:4 ab	6:10 b	14:2 cd	10:6 bc	11:5 bc	11:5 cde
1379	16:0 b	4:12 ab	15:1 d	15:1 c	13:3 c	14:1 e

Pairs of values represent with: without fungus.

Within each column, values with the same letter are nsd(p=>0.05) using X^2 analysis and Fisher's Exact Test.

*Reisolaton of the pathogen from bulb stem tissue was conducted (2.8.5) 7 months post inoculation.

Isolates F_3 and Y_1 generally did not induce significant wilt symptoms in WST cross but R_1 and 1379 significantly reduced growth (Table 2 a) and caused extensive leaf chlorosis and necrosis (Table 2 b). Reisolation from bulb tissue showed that the majority of inoculated palms contained the pathogen.

Inoculation of WR cross 1 with these four isolates showed that this cross was generally unaffected by R_1 and Y_1 with regard to external symptoms (Tables 2 a and b) as well as internal symptoms (Table 2 c) but these isolates were reisolated from the bulbs of some

inoculated palms (Table 2 d). However, by comparison, isolates F_3 and 1379 significantly reduced growth (Table 2 a), induced vascular browning in the bulb (Table 2 c) and were frequently reisolated from bulb tissue of inoculated plants (Table 2 d).

Severe wilt symptoms were observed when WR cross 2 was inoculated with isolates F_3, Y_1 and 1379. Significant chlorosis and necrosis (Table 2 b) occurred with Y_1 and 1379 and all the three isolates induced significant vascular necrosis (Table 2 c) and were consistently reisolated from inoculated palms. Isolate R_1, however, did not induce as many symptoms in this cross; although some vascular browning was observed this was not significant and only 50% of inoculted plants contained the fungus.

Table 2e: Comparative resistance of oil palm lines and aggressiveness of fungal isolates.

	ISOLATES			
OIL PALM LINE	**F_3**	**Y_1**	**R_1**	**1379**
WS CROSS 1	13:3 a	11:5 a i	12:4 a i	16:0 a i
WS CLONE 1	11:4 a	12:3 a i	11:3 a i	15:1 a i
WST CROSS	7:9 a	3:13 b i	12:4 a i	15:1 a i
WR CROSS 1	11:5 a	4:12 b i	3:13 b i	13:3 a ii
WR CROSS 2	10:6 a	13:3 a ii	8:8 ab i	14:1 a ii
WR CLONE 2	7:9 a	3:13 b i	2:14 b i	5:11 b i

Ratios represent with: without vascular necrosis.

Within each column, values with the same letter are nsd.

Within each row, values with the same number are nsd .(X^2 analysis and Fisher's Exact Test) ($P>0.05$).

When resistance was compared in all hostlines inoculated with isolates Y_1, R_1, F_3 and 1379 (Table 2 e) the results revealed that there were no significant differences in the resistance

of any palm line when isolate F_3 was used but significant differences occurred when the other three isolates were used. WR clone 2 (UF 28) was consistently the most resistant palm line and was confirmed and thereafter considered as the wilt resistant standard against which all other material could be compared; anything significantly different from this clone was designated as susceptible while lines not significantly different from this clone were designated resistant (Table 2 f).

Table 2f: Comparative resistance of oil palm lines to 3 fungal isolates.

	ISOLATES		
OIL PALM LINE	**Y_1**	**R_1**	**1379**
WS CROSS 1	S	S	S
WS CLONE 1	S	S	S
WST CROSS	R	S	S
WR CROSS 1	R	R	S
WR CROSS 2	S	S/R	S
WR CLONE 2	R	R	R

Analysis was conducted on data from Table 2(e).

S indicates that ratio is significantly different from standard resistant, (WR Clone 2).

R indicates that ratio is not significantly different from standard resistant (WR Clone 2).

S/R indicates that ratio is only significantly different from standard resistant (WR Clone 2) is a one way test is used.

Thus, all oil palm lines (with the exception of WR clone 2) would have been regarded as susceptible if they were inoculated with isolate 1379 but if R_1 were used to screen these oil

palm lines , WR cross 1 and WRclone 2 would be considered resistant; WR cross 2 would also have some measure of resistance. WST cross, WR cross 1 and WR clone 2 would also be considered resistant if Y_1 had been used.

Conversely, the comparative levels of aggressiveness among the isolates was also conducted using data in Table 2 e by examining the data in the rows. Isolate F_3 was not used in the analysis because it failed to reveal significant differences between different palm lines but Y_1 R_1 and 1379 were used in this comparison. With the extreme susceptible lines (WS cross 1 and WS clone 1) and the most resistant line (WR clone 2) there was no significant difference in levels of aggressiveness of these isolates. However, isolate R_1 was significantly different from 1379 when WR cross 2 was inoculated and isolate Y_1 was intermediate between the two, but the most significant differences were demonstrated with the wilt standard cross and WR cross 1. Thus, with WR cross 1, isolate 1379 caused significantly more disease ($p = < 0.004$) than the Zairean isolates, but this may be explained in terms of the overall greater aggressiveness of 1379. However, with the WST cross, isolates R_1 and 1379 produced similar levels of disease which was significantly more ($p = < 0.004$) than the level induced by Y_1. In all other host material Y_1 and R_1 were similar in aggressiveness.

In summary, results obtained here have revealed differences in levels of aggressiveness of isolates from within and between regions and for the first time these data indicated differential interactions between isolates of *F.o.e.* and oil palm lines. Soil isolates induce few symptoms and to some extent appear to promote growth. In addition, extreme WS

and WR oil palm material has been identified from the material available at the time. This material will be used for detailed studies of this host/pathogen interaction (section 4).

3.2.2 Aggressiveness of nine isolates of *F.o.e.* on two oil palm clones of extreme resistance and susceptibility.

Results from previous experiments have important implications in release of palms that have been identified as resistant using one or a few pathogenic isolates, which may encounter other *F.o.e.* isolates in other areas/countries that are more aggressive or virulent than the ones against which it was originally tested. WR resistant and WS oil palm lines identified in experiment 1 were now further tested using nine pathogenic strains (including F_3 and 1379) isolated from countries where oil palm is grown.

Fourteen replicate plants of two clonal oil palm lines WR (UF 28) and WS (UF177) were inoculated (2.3).with nine isolates of *F.o.e.* namely F_3 (Zaire), Lobe (Cameroon), N1, Abak, OPC_4 (Nigeria), 16F and 146F (Ivory Coast), 1379 (Brazil) and E_1 (Ecuador). Disease development and reisolation were assessed as previously described (2.8.1 - 2.8.5).

Following inoculation, the WS clone exhibited severe stunting (Table 3 a) significant leaf chlorosis and necrosis (Table 3 b) and vascular browning of the bulb (Table 3 c). The fungus was reisolated from the majority of the inoculated plants (Table 3d).

Table 3a: Growth* of WS and WR clones folowing inoculation.

ISOLATES	WS CLONE	WR CLONE
F_3	56.3 b	95.1 a
Lobe	54.1 b	89.5 a
N_1	53.4 bc	91.3 a
Abak 1746	49.2 bc	96.3 a
OPC_4	61.5 ab	98.2 a
146	49.7 bc	66.7 b
16F	43.6 c	74.7 b
1379	41.3 c	80.5 ab
E_1	54.4 b	87.9 ab
Control	69.1 a	90.6 a

Values represent the mean of 14 replicates.

Within each column, values with the same letter are nsd ($p>0.05$) using Kruskal Wallis and repeated U tests.

*Height (cms) see Table 2(a)

It was thus confirmed that, all isolates tested were pathogenic and the isolates from Ivory Coast and Brazil were particularly aggressive. However, the Ivory Coast isolates also induced very severe stunting, widespread leaf chlorosis and necrosis in the WR clone (Tables 3 a and b). In addition, significant vascular necrosis was caused by Ivory Coast, Brazilian and Zairean isolates (Table 3 c). The fungus was also reisolated from many of the plants inoculated with isolates from Ivory Coast, Zaire and Brazil (Table 3 d). Thus,

for the first time this WR standard clone was shown as susceptible to Ivory Coast isolates and to some extent to the Brazilian and Zairean isolates.

Table 3b: External symptoms exhibited by WS and WR clones following inoculation

ISOLATES	WR CLONE	WS CLONE
F_3	1.3 a	2.4 b
Lobe	1.3 a	2.3 b
N_1	1.5 a	2.1 b
Abak 1746	1.2 a	2.8 b
OPC_4	1.2 a	2.4 b
146	2.9 b	3.9 c
16F	2.2 b	4.0 c
1379	2.1 ab	3.9 c
E_1	1.3 a	2.6 b
Control	1.2 a	1.3 a

Values represent a mean of 14 replicates.

Wilt index is based on disease score 0-5 (Flood *et al.*, 1989).

Within each column, values with the same letter are nsd ($p>0.5$) using Kruskal Wallis and repeated U tests.

Table 3c: Internal symptoms exhibited by two inoculated WS and WR clones.

ISOLATES	WS CLONE	WR CLONE
F_3	13:1 bc	6:8 bcd
Lobe	11:3 bc	2:12 abc
N_1	9:5 b	1:13 ab
Abak 1746	14:0 C	2:12 abc
OPC_4	12:2 bc	4:10 abcd
146	14:0 c	8:6 d
16F	14:0 c	6:8 bcd
1379	14:0 c	7:7 cd
E_1	12:2 bc	3:11 abcd
Control	0:14 a	0:14 a

Table 3d: Reisolation of *F. oxysporum* from infected bulb tissue of two clones (WS and WR) following inoculation .

ISOLATES	WS CLONE	WR CLONE
F_3	13:1 bc	6:8 bcd
Lobe	11:3 bc	2:12 abc
N_1	9:5 b	1:13 abc
Abak 1746	14:0 c	2:12 abc
OPC_4	12:2 bc	3:11 abcd
146	14:0 c	8:6 d
16F	14:0 c	6:8 bcd
1379	14:0 c	6:8 bcd
E_1	12:2 bc	3:11 abcd
Control	0:14 a	0:14 a

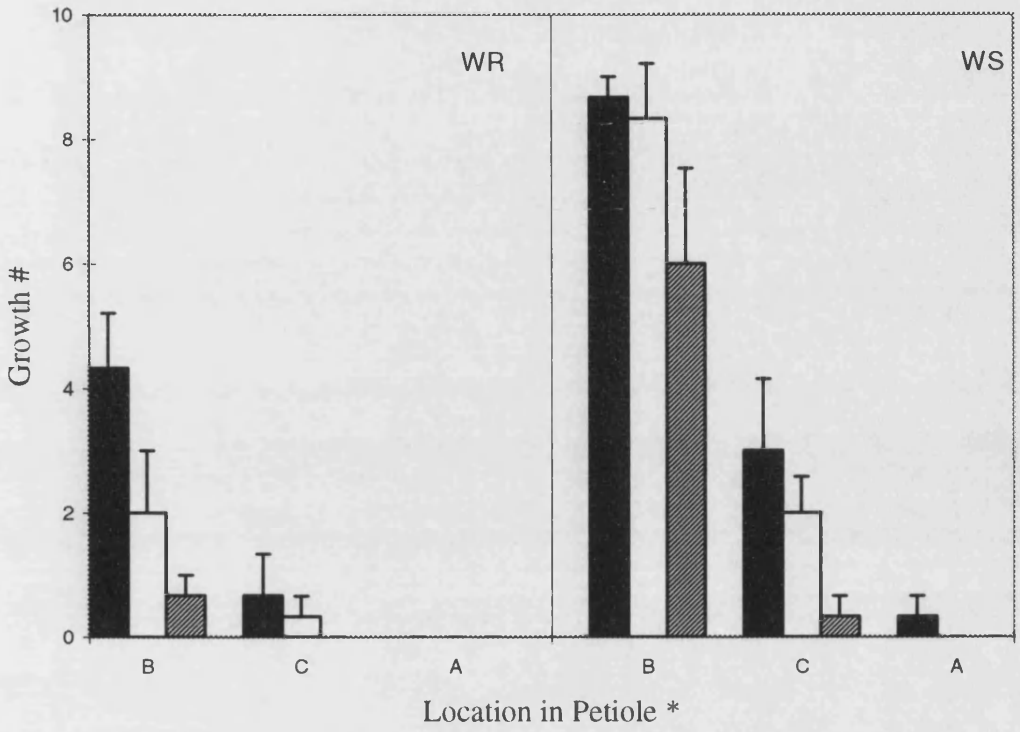
Ratios represent with: without vascular necrosis (Table 3c)or fungus (Table 3d).

Within each column, values with the same letter are nsd (X^2 analysis and Fisher's Exact Test) ($p>0.05$).

3.2.3. Distribution of three pathogenic isolates of *F.o.e.* in rachis of WR and WS oil palm clones.

The progress of infection by *F.o.e.* in oil palm leaves measured as external symptoms was investigated in relation to varietal resistance/susceptibility and to distribution of the fungus in leaves from palms that had been inoculated for seven months. Reisolation was conducted with leaves from severely infected palms. Each leaf was sampled at the base or rachis (point of attachment to the bulb), middle and proximal end of petiole (1 g per section). Each section was comminuted separately and dilution series prepared and plated as previously described (2.8.5), then colony forming units determined. Results from reisolation of. *F.o.e.* from infected oil palm leaves revealed that both clones yielded the fungus from rachis and midpetiole locations. Highest number of propagules were obtained from the basal segment of the rachis with more in WS clone (Fig. 1). There appeared to be a general decline in the amount of fungus reisolated with distance from the base in both clones such that at the proximal end of the petiole the fungus was low or absent (Fig. 1). The distribution of the most pathogenic isolates (16F and 1379) in both clones coincided with the results obtained at the end of nursery trials, where these two isolates were most frequently reisolated from infected bulb tissue. There was, however, some indication of varietal difference in the overall greater amounts of fungus reisolated from WS clone compared to the resistant clone (Fig. 1).

Figure 1. Distribution of three pathogenic isolates of *F.o.e.* in rachis of WR and WS oil palm clones.



* Samples were taken from the base (B) centre (C) and apex (A) of the petiole.

cfu's x 10^{-3} g^{-1} fresh wt.

Values are the mean (±SE) of three observations. Isolates 16F, 1379 and F_3 are represented by filled, open or hatched columns respectively.

The data so far presented confirmed that all the isolates tested were pathogenic to susceptible oil palm. Ivory Coast isolates were highly aggressive and induced severe stunting, chlorosis and necrosis on a material which had been selected for resistance to Zairean isolates. Similarly the Brazilian isolate was shown to be more aggressive than the Zairean isolate. The most aggressive isolate penetrated both clones and systemically

colonized plants as revealed by reisolation from both bulbs and leaf stalks (rachis and petioles). Susceptible plants contained more fungus than resistant plants. Although the fungus was reisolated from the leaves, distribution appeared to be concentrated at the basal rachis and to some extent midpetioles.

3.2.4 Virulence and aggressiveness of three African isolates on 14 oil palm clones.

In the previous experiments (3.2.1) it was suggested that some form of genetic interaction occurred when a wilt standard cross appeared to be susceptible to *F.o.e.* isolate R_1 but resistant to Y_1. This experiment was planned to be repeated with more replicate plants but due to unavailability of plant material (seedling crosses) from Africa, plantlets from 14 different clones were supplied and inoculated with three African *F.o.e.* isolates (Abak 508.1746, 16F and $F_{3)}$. These isolates were used in nursery screening trials in Nigeria, Ivory Coast and Zaire respectively. Inoculum production and inoculation of plantlets and disease assessments were done as previously.

Table 4a: Comparison of isolate aggressiveness

ISOLATE	FREQUENCY OF INFECTION (%) #	% BROWNING OF BULB*	DISEASE*
F_3	39.7 a	55.2 b	1.78 a
16F	65.3 b	64.6 c	2.89 b
Abak	59.4 b	45.6 a	1.49 a

#Values are the mean of 14 clones with 17-24 palms per clone.

Browning of bulb tissue indicates infection.

*Values are the mean of all infected plants.

Within each column, values with the same letter are not significantly different (P>0.01, Kruskall Wallis and Mann Whitney U-Test

The Ivory Coast isolate (16F) and the Nigerian (Abak 508.1746) isolate were similarly aggressive in terms of the number of inoculated palms that became infected. Both isolates produced significantly more infected palms than the Zairean isolate F_3 (Table 4 a). Plants inoculated with isolate 16F exhibited significantly more foliar chlorosis and necrosis and extensive vascular browning than the other two isolates. Isolate F_3 induced a similar level of leaf symptoms but significantly more internal symptoms than Abak 508.1746 (Table 4a)

Generally, there was a very good correlation observed between isolates when percentage of inoculated palms that became infected in different clones was assessed (correlation coefficient, F_3 *vs* Abak $r = 0.849$, significant at $P < 0.01$) (Table 4b).

Nonetheless, there were some differences observed amongst some clones whereby ranking for 16F was different to that for F_3 and Abak (Table 4 b). For example clone 3 appeared to be susceptible to 16F (79% infection) and yet relatively resistant to F_3 and Abak isolates (35% and 41% respectively). On the other hand, clone 7 showed (to a lesser extent) a reverse of this effect. However, the overall analysis of results showed no significant clone/isolate interaction ($P > 0.05$, generalised linear regression of a binominal model of frequency of infection, Genstat 5 release 2.2). Nevertheless, if clone 1 were used as standard resistant and any clone with a significantly higher infection was defined as susceptible, then clones 3 and 7 would vary in their resistance rating depending on the isolate used.

Table 4b: A comparison of resistance of 14 clones.

ISOLATE					
F_3		16F		Abak	
Clone	**With:without infection**	**Clone**	**With:without infection**	**Clone**	**With:without infection**
1	2:22a	1	5:19a	1	5:19a
UF28	5:18ab	7	11:12ab	3	9:13ab
2	6:16ab	UF28	12:11ab	UF28	12:12abc
3	8:15abc	6	13:11bc	2	11:10abc
4	7:12abcd	9	13:8bcd	4	12:9bcd
5	8:13bcd	8	14:6bcd	4	13:10bcd
6	10:13bcd	4	12:5bcde	10	12:8bcd
7	10:13bcd	10	13.5bcde	7	14:8bcd
8	9:10bcd	2	15:5bcde	6	15:8bcde
9	10:11bcd	3	19:5bcde	9	15:7bcde
10	10:10bcd	12	16:4bcde	13	15:6bcde
11	9:8bcd	5	18:3cde	5	16:5cde
12	13:7bcd	11	11 14:1de	12	18:3de
13	14:6d	13	13 21:1e	11	17:1e

For each isolate, clones are ranked from the most resistant to the susceptible.

Within each column, values with the same letter are not significantly different (Chi-squared and Fishers Exact Test P>0.05).

UF28 is a standard resistant clone.

In summary, a comparison of three isolates of *F.o.e.* from different regions in West and Central Africa were compared using 14 oil palm clones revealed that the resistance ranking

of some clones varied with different isolates but overall the results showed no significant isolate/clone interaction. A good correlation between the frequency of infection and the severity of symptoms in infected palms was shown with Nigerian and Ivory Coast isolate.

3.2.5 Effect of soil isolates on WS oil palm clones

Results from an earlier experiment (3.2.1) showed that isolates from Malaysia (LEY), Cameroon (CAM) and Zaire (BOS) did not induce significant symptoms in any oil palm line tested despite being reisolated from bulb tissue. It was also observed that these soil isolates rather than reducing growth appeared to slightly promote growth of most oil palm lines when compared to the controls.

To investigate this finding further, an experiment was conducted in which WS clone1 was inoculated (2.3) with 7 soil isolates from oil palm plantations in West and Central Africa plus one pathogenic isolate (F_3) from Zaire as a positive control. Disease assessments were conducted as above (2.8.1-2.8.5).

All soil isolates failed to induce any wilt symptoms on WS clone (Table 5). Reisolation data (not shown) revealed only 1 - 2 plants (6-12%) in each treatment contained the fungus in the bulb tissue. It was again observed that these isolates slightly promoted growth but the growth effect was not significant. However, F_3, the only pathogenic isolate in the experiment induced significant leaf chlorosis and vascular browning in the bulb and reduced growth although again the reduction in growth was not significant. Thus, isolates of *F. oxysporum* from soil generally appeared to be avirulent to oil palm.

Table 5a: Growth, external and internal symptoms of WS Clone 1 following inoculation.

Isolate	Height (cm)	Leaf chlorosis/ Necrosis	With:without browning in the bulb
IC_3	120.3 (ab)	1.0 (a)	0:2 (a)
F_2 4844	118.7 (ab)	1.3 (a)	0:12 (a)
IC_9	118.3 (ab)	0.8 (a)	0:12 (a)
IC_5	114.9 (ab)	0.8 (a)	0:12 (a)
G_4	111.9 (ab)	1.3 (a)	0:12 (a)
G_1	111.8 (ab)	1.3 (a)	0:12 (a)
C_2	110.9 (ab)	1.9 (a)	0:12 (a)
F_3	94.1 (a)	2.2 (b)	8:4 (b)
Control	109.0 (a)	0.6 (a)	0:12 (a)

Values represent a mean of 12 replicates.

Within each column, values with the same letter are nsd ($P>0.05$) using Kruskal Wallis and repeated U-Tests.

[Wilt index or leaf chlorosis based on disease score (Flood *et al,* 1989)]1 - 2.8.5).

3.2.6 Inoculation of putative resistant oil palm crosses with aggressive *F.o.e.* isolates obtained world wide.

Following observation of variations in the levels of aggressiveness of the *F.o.e.* isolates from different regions and observations that material selected for resistance in Nigeria was susceptible when planted in Ivory Coast, and vice versa, (Renard, 1991 per. comm.; Oritsejafor, 1989), it was decided to conduct a series of experiments comparing oil palms

selected for resistance in Zaire, Ivory Coast and Nigeria. However, delays in obtaining plant material from Africa, resulted in experiments being conducted separately. Also, the number of isolates and experimental replication used were restricted by number of plants available.

3.2.6.1 Inoculation of seedling material selected for resistance in Zaire.

Seeds of two 'Dumpy' oil palm progenies (100% and 75% extent of selfing) selected for extreme wilt resistance in Zaire were treated and grown as described previously (2.1). These Dumpy progenies were inoculated (2.3) with eight aggressive isolates F_3, Y_1, 1379, E_1, 16F, 146F, Abak 508.1746, Ndian 3AR4 and a representative soil isolate G4.

Table 6a: Growth$^+$ of two Dumpy crosses (WR) from Zaire following inoculation .

ISOLATES	100%* DUMPY	75%* DUMPY
1379	80.9 c	77.3 a
Y_1	71.0 bc	
F_3	68.7 b	80.6 a
E_1	65.7 b	
Abak 1746	63.4 ab	81.9 a
16F	57.1 a	46.8 b
G4	57.0 a	
Ndian AR4	49.5 a	
146	48.9 a	
Control	57.0 a	82.2 a

Values represent the mean of 16 replictes

Within each column, values all the same letter and nsd ($p>0.05$) using Kruskall Wallis and repeated U tests.

*100/75% denote extent of selfing.

†Height (cm) see table 2 (a)

Table 6b: External symptoms exhibited by two Dumpy crosses (WR) from Zaire following inoculation.

ISOLATES	100%DUMPY	75% DUMPY
1379	0.5 a	0.6 a
Y_1	1.0 a	
F_3	0.8 a	0.9 a
E_1	1.0 a	
Abak 1746	0.9 a	0.7 a
16F	1.4 a	3.1 b
G4	1.1 a	
Ndian AR4	1.2 a	
146	1.6 a	
Control	1.0 a	0.5 a

Values represent a mean of 16 replicates

Wilt index is based on a disease score 0-5 (Flood *et al.*,1989).

Within each column, values with the same letter are nsd ($p>0.5$) using Kruskal Wallis and repeated U tests.

One hundred percent Dumpy material showed very limited external symptoms with no significant reduction of height and few internal symptoms (Table 6a and b). On the contrary, a significant increase in height was observed in most inoculated plants (Table 6 a). However, with 75% Dumpy, the Ivory Coast isolate (16F) induced significant stunting (Table 6 a) and leaf chlorosis and necrosis (Table 6 b). This isolate, 16F, also induced

significant internal vascular browning in the bulb in both 100% and 75% Dumpy material (Table 6 c) and was reisolated most frequently from the bulb tissue (Table 6 d). Similarly, the Brazilian isolate (1379) and the Nigerian isolate (Abak 508.1746) also induced vascular browning in bulb tissue in 75% Dumpy compared to (F_3) the Zairean isolate (Table 6 c) and these isolates were reisolated more frequently than F_3 (Table 6 d). The Ghanaian soil isolate (G4) did not induce any wilt symptoms in 100% Dumpy and could not be reisolated (Tables 6 a-d).

Table 6c: Internal symptoms exhibited by two Dumpy crosses (WR) from Zaire following inoculation.

ISOLATES	100% DUMPY	75% DUMPY
1379	3:13 bc	8.8 bc
Y_1	3:13 bc	
F_3	2:14 ab	2:14 a
E_1	4:12 bc	
Abak 1746	2:14 ab	7.9 b
16F	7:9 c	13:3 c
G4	0:16 a	
Ndian AR4	4:12 bc	
146	4:12 bc	
Control	0:16 a	0:16 a

Ratios represent with:without vascular necrosis (P=>0.05)

Within each column, values with the same letter are result X^2 analysis and Fisher's Exact test.

Table 6d: Reisolation of *F. oxysporum* from infected bulb tissue of two WR Dumpy crosses from Zaire.

ISOLATES	100% DUMPY	75% DUMPY
1379	3:13 bc	8:8 bc
Y_1	3:13 bc	
F_3	2:14 ab	2:14 a
E_1	4:12 bc	
Abak 1746	2:14 ab	7:9 b
16F	7:9 c	13:3 c
G4	0:16 a	
Ndian3 AR4	4:12 bc	
146	4:12 bc	
Control	0:16 a	0:16 a

Pairs of values represent with:without fungus.

Within each column, values with the same letter are nsd (P=>0.05) using X^2 analysis and Fisher's Exact test.

3.2.6.2 Inoculation of seedling material selected for resistance in Ivory Coast

Seedlings from four crosses originating from Ivory Coast and designated as 2 WR (a and b) and 2 WS (c and d) in Ivory Coast were grown (2.1) and inoculated (2.3) with isolates from Zaire (F_3, Y_1 and R_1), Ivory Coast (16F), Brazil (1379) and Nigeria (Abak 508.1746).

Table 7a:Growth* of four crosses originating from Ivory Coast following inoculation

ISOLATE	CROSS (a)	CROSS (b)	CROSS (c)	CROSS (d)
Y_1	103.4 ab	102.3 b	89.0 ab	86.8 a
R_1	106.7 ab	91.7 d	92.5 a	84.7 ab
Abak 508.1746	92.3 bc	93.0 cd	82.0 bc	80.1 ab
F_3	109.1 a	118.0 a	95.3 a	67.7 b
1379	94.4 bc	97.6 bcd	76.3 cd	47.4 c
16F	80.4 c	86.0 d	64.0 d	50.9 c
Control	109.4 a	99.6 bc	87.8 ab	80.0 ab

Values represent a mean of 11-16 replicates

Within each column, values with the same letter are nsd (P>0.05) using Kruskal Wallis and repeated U tests.

*Height (cm) see table 2 (a)

Table 7b: External symptoms exhibited by four crosses from Ivory Coast following inoculation.

ISOLATES	CROSS (a)	CROSS (b)	CROSS (c)	CROSS (d)
Y_1	0.8 a	0.5 a	0.9 a	1.0 a
R_1	0.6 a	0.5 a	0.5 a	0.8 a
Abak 508.1746	1.1 a	0.5 a	0.6 a	1.1 a
F_3	0.9 a	0.5 a	0.6 a	1.6 a
1379	1.3 a	0.5 a	1.5 ab	3.0 b
16F	1.5 a	1.0 a	2.1 b	3.2 b
Control	1.0a	0.5 a	0.6 a	1.0 a

Values represent a mean of 11-16 replicates.

Wilt Index is based on disease score 0-5 (Flood *et al.*,1989).

Within each column values with the same letter are nsd (P>0.05) using Kruskal Wallis and repeated U Tests.

WS crosses (c) and (d) exhibited severe stunting (Table 7 a), wide spread leaf chlorosis and necrosis (Table 7 b) with 16F and the Brazilian isolate. Many of these plants inoculated with 16F and 1379 had significant vascular browning in the (Table 7 c). The Zairean isolate Y_1 also induced significant vascular browning in the bulb in this cross (c) (Table 7 c). These isolates were also reisolated more frequently in crosses (c) and (d) than the other isolates (Table 7 d). With WR cross (b) only 16F and R_1 significantly reduced growth (Table 7 a), while F_3 and Y_1 promoted growth. However, despite R_1 reducing growth, little leaf chlorosis or necrosis was observed (Table 7 b) and it failed to induce vascular browning in the bulb (Table 7 c). Only 16F was frequently reisolated from cross (b). With regards to cross (a), Ivory Coast, Brazilian and Nigerian isolates caused significant reduction in growth (Table 7 a) and 16F and 1379 induced severe vascular necrosis in the bulb tissue (Table 7 c) but failed to cause foliar chlorosis or necrosis (Table 7 b). Vascular browning (Table 7 c) was observed to some extent with all isolates but notably by Y_1, Abak, 16F and 1379 and similarly the Brazilian and Ivory Coast isolates were most frequently reisolated (Table 7 d).

Despite being designated as WR, cross (a) was susceptible to the Ivory Coast and Brazilian isolates when considering internal symptoms and reisolation of the pathogen but this cross exhibited very few external symptoms. Similarly, WR cross (b) exhibited few external symptoms even after inoculation with 16F.

Table 7c: Internal symptoms exhiited by four crosses originating from Ivory Coast following inoculation.

ISOLATES	CROSS (a)	CROSS (b)	CROSS (c)	CROSS (d)
Y_1	2:12 a	1:10 a	10:5 bc	11:5 a
R_1	6:8 ab	0:11 a	3:12 a	13:3 ab
Abak 508.1746	6:8 ab	2:9 ab	7:8 ab	13:3 ab
F_3	4:10 ab	3:8 ab	4:11 a	12:4 a
1379	9:5 bc	3:8 ab	11:4 bc	16:0 b
16F	12:2 c	6:5 b	14:1 c	16:0 b
Control	0:14 a	0:11 a	0:15 a	0:16 a

Ratios represent with:without vascular necrosis (P>0.05).

Within each column, values with the same letter are result X^2 analysis and Fisher's Exact t

Table 7d: Reisolation of *F.oxysporum.* from infected bulb tissues of four crosses from Ivory Coast.

ISOLATES	CROSS (a)	CROSS (b)	CROSS (c)	CROSS (d)
Y_1	2:12 a	1:10 a	10:5 bc	11:5 a
R_1	6:8 ab	0:11 a	3:12 a	13:3 ab
Abak 508.1746	6:8 ab	2:9 ab	7:8 ab	13:3 ab
F_3	4:10 ab	3:8 ab	4:11 a	12:4 a
1379	9:5 bc	3:8 ab	11:4 bc	16:0 b
16F	12:2 c	6:5 b	14:1 c	16:0 b
Control	0:14 a	0:11 a	0:15 a	0:16 A

Pairs of values represent with:without fungus.

Within each column, values with the same letter are nsd (P=0.05) using X^2 analysis and Fisher's Exact test.

3.2.6.3 Inoculation of seedling crosses selected for resistance in Nigeria

Oil palm seeds from Nigerian Institute for Oil Palm Research (NIFOR) that had been selected and bred for resistance in Nigeria (crosses (1) and (2)) were grown (2.1) and inoculated (2.3) with 15 isolates that included F_3, Y_1, and R_1 and Binga (Zaire); Abak 508.1746, Abak 508.1322, OPC_1, OPC_4 (Nigeria); Ndian 3AR4, Ndian C_{10}, Lobe2 and Cope 1 (Cameroon); E_1 (Ecuador); 1379 (Brazil) and 16F (Ivory Coast). Due to unavailability of susceptible crosses, WS clone (UF177) was supplemented.

The majority of the isolates caused significant reduction of growth, and wide spread vascular necrosis in the bulb in cross (1) (Tables 8 a - c). Although few external symptoms were apparent in most inoculated plants, some isolates induced significant chlorosis/necrosis. Cross (1) was particularly susceptible to 16F, 1379, Cope 1, R_1 with regard to external and internal symptoms (Tables 8 a - c). Similarly, with cross (2) the majority of the isolates induced significant external and internal symptoms. Isolates 16F, 1379 were again particularly aggressive and to some extent so were isolates E_1, F_3 and Abak 508.1746 (Tables 8 a - c). All the isolates with the exception of OPC_1 caused significant wilt symptoms in WS clone UF177.

Table 8a: Growth* of two Nigerian crosses (WR) and one clone (WS) following inoculation.

	OIL PALM LINES		
ISOLATES	**CROSS (1)**	**CROSS (2)**	**WS CLONE (UF177)**
OPC_1	95.9 a	86.9 a	68.9 a
F_3	91.3 a	71.8 cde	55.7 b
Ndian C_{10}	90.5 ab	71.6 cde	52.5 b
Lobe 2	82.8 bc	83.8 ab	53.6 b
Ndian 3AR4	82.9 bcd	77.6 abcd	50.3 bc
Abak 5081322	81.7 bcd	73.2 abcd	49.1 bc
OPC4	80.9 bcd	81.1 abc	54.8 b
Binga	80.4 bcd	75.6 bcd	51.6 b
Y_1	79.9 bcd	74.2 bcde	52.4 b
E_1	79.4 bcd	61.9 e	56.1 b
Abak508.1746	74.4 cd	68.9 de	48.6 bc
R_1	74.3 cd	74.0 bcde	49.3 bc
Cope 1	70.8 cde	83.6 ab	47.0 bc
1379	69.4 de	69.3 de	40.4 c
16F	58.3 e	48.7 f	42.5 c
Control	96.0 a	87.9 a	70.2 a

Values represent a mean of 16 replicates.

Within each column, values with the same letter are nsd ($P>0.05$) using Kruskal Wallis and repeated U-tests.

*For each cross growth is the mean height (cm) of fully expanded youngest leaves for each treatment measured from soil level to the tip of the leaf at 28 d in tervals from 3-7 months post inoculation.

Table 8b:External symptoms exhibited by two Nigerian Crosses (WR) and one WS clone following inoculation.

	OIL PALM CROSSES		
ISOLATES	**CROSS (1)**	**CROSS (2)**	**WS CLONE (UF177)**
OPC_1	0 a	0 a	0 a
F_3	0.3 a	2.1 d	2.2 b
$NdianC_{10}$	0.06 a	0.9 bcd	1.8 b
Lobe 2	0.2 a	0 a	2.1 b
Ndian3AR4	0.8 ab	0.7 ab	2.1 b
Abak5081322	0.4 a	0.5 ab	1.9 b
OPC4	1.06 ab	0.2 a	2.3 b
Binga	0.6 ab	1.1 bcd	2.0 b
Y_1	0.9 ab	0.9 bcd	1.8 b
E_1	0.8 ab	2.1 d	3.0 b
Abak 508.1746	0.9 ab	1.6 cd	3.3 b
R_1	1.6 b	1.2 bcd	2.3 b
Cope I	1.4 b	0.8 abc	1.9 b
1379	1.3 b	1.8 cd	3.8 c
16F	2.9 c	3.6 e	4.3 c
Control	0 a	0.2a	0 a

Values represent a mean of 16 replicates.
Wilt Index is based on disease score 0-5 (Flood *et al.*, 1989).
Within the same column, values with the same letter are nsd ($P>0.05$) using Kruskal Wallis and repeated U tests.

Table 8c: Internal symptoms exhibited by two Nigerian crosses (WR) and one WS clone following inoculation.

	OIL PALM LINES		
ISOLATES	**CROSS (1)**	**CROSS (2)**	**WS CLONE**
OPC_1	0:16 a	0:16 a	0:16 a
F_3	4:12 abc	8:8 bc	14:2 c
Ndian C_{10}	1:15 a	1:15 a	2:14 a
$Lobe_2$	2:14 ab	6:10 bc	13:3 bc
Ndian 3AR4	7:9 cd	9:7 bc	10:6 b
Abak 508.1322	9:7 cd	6:10 bc	15:1 c
OPC_4	9:7 cd	6:10 bc	15:1 c
Binga	4:12 abc	6:10 bc	14:2 c
Y_1	6:10 bcd	4:12 ab	13:3 c
E_1	7:9 cd	15:1 d	14:2 c
Abak568.1746	6:10 bcd	9:7 bc	16:0 c
R_1	11:5 de	10:6 c	13:3 c
Cope 1	7:9 cd	6:10 bc	14:2 c
1379	9:7 cd	11:5 cd	16:0 c
16F	13:3 e	15:1 d	16:0 c
Control	0:16 a	0:16 a	0:16 a

Ratios represent with:without vascular necrosis (P=>0.05).

Within each column, values with the same letter are result X^2 analysis and Fisher's Exact test.

To summarise this series of experiments, the Zairean crosses exhibited few external symptoms when inoculated with the aggressive isolates except 75 % Dumpy when it was inoculated with isolate 16F. However, all the isolates tested induced significant internal symptoms except the Zairean isolate F_3, the Nigerian isolate ,Abak and G_4 .

The Ivory Coast crosses (a) and (b) exhibited few symptoms following inoculation with these aggressive isolates with the exception of the two isolates, 16F and 1379. Severe

vascular necrosis was induced in all the four crosses inoculated with the majority of isolates ;16F and 1379 were frequently reisolated from inoculated palms even from cross (b.)

The Nigerian cross (1) exhibited few external symptoms with the majority of isolates; 16F was again particularly aggressive whilst cross (2) exhibited wide spread external symptoms when inoculated with aggressive isolates. Most of the isolates induced significant vascular necrosis in the two crosses especially isolate 16F.

3.2.7.Comparison of pathogenicity of *Fusarium oxysporum* f. sp. *albedinis* and *Fusarium oxysporum* f. sp. *elaeidis.*

In countries with a diversity of climatic variations such as Nigeria and Sudan both oil palm and date palm are grown as important cash crops. Oil palm is grown in wet and humid areas whilst dates grow in arid or semi arid hot region. Both national and international trading items from these countries constitute, among other items, palm products. Since the organisms responsible for causing wilt diseases to these crops can be transported and preserved for a long period of time in soil, in seeds as in oil palm (Locke and Colhoun, 1974; Flood *et al.,* 1994) and in palm fragments used in the production of craft items (Djerbi, 1982), there would appear to be a potential risk for the pathogen of one crop infecting the other. This experiment was conducted to investigate this possibility.

Seeds from susceptible (Deglet Noor) and resistant (Medgool) date palm cultivars were surface sterilized in sodium hypochlorite (10% v/v) for ten minutes before rinsing twice in sterile distilled water. These seeds were wrapped in moist sterile tissue paper and

incubated at 28°c to initiate germination. After germination, both date palm and corresponding (WS and WR) oil palm materials were grown and inoculated as before (2.1; 2.3) with isolates 1379, F_3 and *Fusarium oxysporum albedinis (F.o.a.).*

External symptoms were not observed in date palms and hence only internal symptoms were considered for disease assessment (2.8.4). Following inoculation of date palm with *F.o.e.* and *F.o.a.,* both isolates induced significant vascular browning in the bulb in both cultivars tested. The Brazilian isolate (1379) caused the most severe vascular necrosis in the two date palm cultivars and *F.o.a.* in the resistant Medgool line (Table 9). However, WR oil palm clone appeared to be susceptible to *F.o.e.* but resistant to the *F.o.a.* isolates. The susceptible oil palm clone was apparently susceptible to both date and oil palm isolates (Table 9). The fungus was reisolated from pseudobulb of all infected plants.

Table 9:Internal symptoms in date and oil palm pseudobulbs inoculated with *F.o.e.* and *F. oxysporum* f. sp. *albedinis (F.o.a.)*

	DATE PALM LINES		OIL PALM LINES	
ISOLATE	**Medgool (WR)**	**Deglet-Nour (WS)**	**WR (clone)**	**WS (clone)**
F_3	3:5 b	4:4 b	2:6 b	8:0 c
1379	6:2 c	5:3 b	6:2 c	7:1 c
F.o.a.	5:3 c	4:4 b	0:8 a	3:5 b
CONTROL	0:8 a	0:8 a	0:8 a	0:8 a

Ratios represent with:without vascular necrosis.

Within each column, values with the same letter are result X^2 analysis and Fisher's Exact test ($P>0.05$).

3.2.8 Investigations of host/pathogen interaction on oil palm callus with a view to develop a rapid screen for resistance.

The immediate aim of these experiments was to develop a rapid method for screening for resistance at a tissue culture level as pathology trials can take six to eight months to complete. Also, the experiment was conducted to investigate if oil palm grown in *vitro* can express resistance to *F.o.e.* In these experiments, calli derived from WR and WS oil palm material were first subcultured onto MS medium and allowed to grow for at least fourteen days before inoculation. These calli were further subcultured, one week prior to inoculation, onto MS medium, tap water agar (poor nutrient medium) or media which had been amended with hormones to induce a more compact structure of callus cells to investigate effect on expression of host response.

3.2.8.1 Fungal colonization on callus grown on Basal Medium (MS) and TWA.

This experiment studied the influence of inoculation method and growth medium. Each experiment consisted of five replicates from each callus line derived from one wilt resistant and two wilt susceptible palm lines (UF28, 177 and 174 respectively).

Callus was inoculated as described previously (2.11.2) using agar blocks and spore suspension. The callus was growm either on MS medium or TWA (low nutrient medium). Controls were inoculated with sterile agar blocks or sterile distilled water. The inoculated calli were incubated at 25 ± 1°c, 8 hr dark and 16 hr light. Daily observations were carried out and the extent of colonization was scored as previously described (2.11.3).

Daily observation and assessment of fungal colonization showed no significant difference between resistant and susceptible clones on either MS medium or TWA medium.. Microscopic examination revealed aerial mycelia and extensive saprophytic growth of the fungus over and between callus cells in both cultivars and at the end of the experiment the fungus had colonized the entire callus and media. However, no penetration of the callus cells was observed. These experiments were repeated several times with similar results.

3.2.8.2 Effects of high and low spore concentrations and callus morphology on fungal colonization of callus

Several attempts to detect expression of resistance to *F.o.e.* in callus lines were unsuccessful. This experiment was therefore planned to investigate if resistance could be expressed when lower spore concentrations were used. Also, by modification of hormonal regime in growth media if the morphological form of callus cells could be altered (loose versus tight callus) that could influence the degree of susceptibility or resistance respectively (Helgeson *et al.* 1972; Holliday and Klarman, 1978).

Two oil palm lines, WR and WS, were subcultured on MS medium supplemented with two concentrations of 2.4.D, + kinetin. Spore concentrations (2.2) were adjusted to 10^6 10^5 and 10^4 spores ml^{-1} and 5 µl droplets were applied to three replicate calli. Controls received sterile distilled water. The calli were incubated as above (2.11.1). Daily observations of fungal colonization were made and colonization was scored as above (2.3.6.2). A final colonization score was made four days post inoculation (Table 10).

Table 10: Fungal colonization of two oil palm callus lines on four different media

	A	B	C	D
WS clone	1.4 ab(i)	1.2 a(i)	1.8 bc (i)	2.2 c(i)
WR clone	2.9 a(ii)	2.8 a(ii)	2.8 a(ii)	2.8 a(i)

Treatment A - 0.2 mg/L 2,4D

Treatment B - 2.0 mg/L 2,4 D

Treatment C - 0.2 mg/L 2,4 D plus 0.2 mg/L kinetin

Treatment D - 2.0 mg/L 2,4D plus 0.2 mg/L kinetin

Values represent mean of nine replicate scores where 0 - no mycelial growth; 1 - mycelial colonization on less than 50 % of callus; 2 - mycelial colonization on 50 - 100% of callus; 4 - mycelial colonization on 100% of callus and media (Holliday & Klarman, 1979).

Within each row, values with the same letter are nsd (p=>0.05) using Kruskal Wallis and repeated U tests. Within each column, values with the same number are nsd (p=>0.05) using Kruskal Wallis and repeated U tests.

In this experiment, the appearance of aerial mycelia was rather slow compared with the previous experiment but mycelial growth from the three different spore concentrations were very similar. Thus the data were pooled to allow further statistical analysis. Colonization of WR line was not affected by manipulation of the hormone balance of the medium (Table 10). However, colonization of WS line appeared to have been influenced by the medium composition which slightly altered the morphological form of callus tissue (loose to tight). Colonization was more extensive where kinetin was present and with the highest concentration of 2.4.D. Colonization of WR line was always greater than that of WS line except in treatment D where the colonization of both lines was similar (Table 10).

It was apparent that despite manipulation of media or spore concentrations, resistance was not expressed at the callus level in this host/pathogen interaction.

3.2.7 DISCUSSION.

The main objective of this section was to screen under standard conditions, oil palm progenies against numerous isolates of *F.o.e.* from the major oil palm growing regions in order to identify very aggressive isolates and select wilt resistant and wilt susceptible palms for further detailed studies on the host / pathogen interaction (Section 4). Inoculation of many oil palm lines with a wide range of fungal isolates may also have demonstrated evidence of a genetic interaction with oil palm and strains of *F.o.e.* In addition, expression of resistance in tissue cultured material was investigated.

None of the soil isolates from different oil palm plantations induced external wilt symptoms in inoculated plants and very little vascular necrosis was observed with these isolates. Mepsted (1992) made similar observations and like this study his observations were based on a limited number of isolates. However, in a test conducted with a large number of soil isolates, Renard (pers. comm., 1991), one isolate in a thousand was shown to be pathogenic.The results presented here suggest that soil isolates in general promote growth and do not appear to induce any external symptoms although the Zairean soil isolate (BOS) did induce some vascular necrosis in the bulb. Ho *et al.*(1985 b) similarly reported that soil isolates could be occasionally reisolated from a few inoculated palms which suggested that the fungus entered, but apparently caused no significant adverse effects on palm tissue.

Matta (1971) suggested that organisms that penetrate the host without causing disease could cause cross protection through a combination of three mechanisms which involve competition for nutrients, competition for infection sites and the induction/enhancement of host defence responses. Recently, Turner (1992) did not observe any external symptoms in oil palm plants inoculated with a soil isolate or with a mixed culture of a soil isolate and a pathogenic isolate. Also, no pathogenic isolate was reisolated from any plant tissue where a mixed culture was applied. In conclusion, she suggested that the addition of an avirulent soil isolate to soil surrounding oil palm seedlngs, could suppress the development of vascular wilt of oil palm. Although not the aim of this current work, further studies on *F.o.e.* soil isolates from oil palm plantations are necessary. For example, vascular wilt has never been reported in Malaysia yet Malaysian isolates do colonize systemically (this study and Flood *et al.,* 1989). It may be that soil isolates occupy an ecological niche that could prevent colonization by the pathogen if it were ever accidentally introduced into Malaysia on contaminated seed (Flood *et al.,* 1990; Flood *et al.,* 1994). Certainly, Park (1958) claimed that soil isolates were better competitors than the pathogenic forms and soil isolates may induce resistance to any subsequent attack by any other aggressive forms as suggested by Taquet *et al.* (1985) and Flood *et al.*(1989).

In the current study, it was demonstrated that *F.o.e.* isolates from infected plants from Zaire, Ivory Coast, Nigeria, Cameroon, Brazil and Ecuador induced severe external and internal wilt symptoms. These results are in agreement with previous reports (Renard *et al.,* 1972; Ho *et al.,* 1985; Obuekwe and Osagie, 1989; Flood *et al.,* 1989). Isolates from Ivory Coast (16F) and Brazil (1379) were particularly aggressive, inducing the most

severe stunting, wide spread leaf chlorosis and necrosis, extensive vascular necrosis in the bulb and were most frequently reisolated from palms. Isolate 16F is known to be a very aggressive isolate used in screening for resistance in Ivory Coast (Renard pers. comm., 1991). Isolate 1379 was isolated from the Denpasa Estate in Brazil and Van de Lande (1983) identified the fungus but did not inoculate healthy plants in order to complete Koch's postulates. The pathogenicity of this isolate to clonal oil palm was subsequently demonstrated (Flood *et al.*, 1989). The Brazilian isolate was recognized as very aggressive, inducing symptoms on material selected for resistance in Zaire to isolate F_3 (Flood *et al.*, 1993) and a similar observation has been made in this study. Isolate 1379 extensively colonized inoculated material and could be reisolated from all parts except the apex of infected plant petioles, 28 weeks after inoculation notably from the leaf bases but never from the chlorotic or necrotic leaves. Ho *et al.*(1985) similarly demonstrated that the fungus was present in the roots, bulb and petioles but never in wilted leaf blades. In contrast *V. dahliae* was frequently isolated from susceptible cotton leaf petioles within three days of basal stem puncture inoculation with all isolates, irrespective of aggressiveness(Garas *et al.*, 1986).

Screening for resistance with several representative isolates from all oil palm growing regions has an advantage over screening with one or a few isolates from selected local areas and should allow logical deployment of plant material in different areas of the world. Such screening programmes will allow a comparison of aggressiveness and /or virulence of a range of isolates from around the world but can only be conducted in an area where oil palm is not grown to avoid introduction of new strains into new areas.

Results presented here and elsewhere (Mepsted *et al.,* 1994) generally revealed that in the reactions of oil palm material to inoculation with different fungal isolates, the degree of aggressiveness varies but there appears to be no change in virulence. These results confirm other early work of (Meunier *et al.,* 1979; de Franqueville, 1984 and Obuekwe and Osagie, 1989). However, there were exceptions to these general conclusions. For the first time, some evidence was observed which revealed that some genetic interaction had occurred between some *F.o.e.* isolates and palm crosses. Seedlings of a wilt standard cross (WSt cross) appeared to be susceptible to isolate R_1 but resistant to Y_1. It was hoped to repeat this experiment using larger numbers of plants but the exact crosses were not available and so the possibility of races existing within *F.o.e.* population could not be tested using this material. However, 14 different clones were available and were inoculated with three isolates which are used in nursery screening trials in West Africa. As before, there were some indications of differences in the ranking of these isolates with some clones but overall there appeared to be no genetic interaction i.e. the existence of races in *F.o.e* was not proven; some of the anomalies of the current study should be investigated further.

The existence of races has already been demonstrated in several form species of *F. oxysporum* including with *F. oxysporum f. sp. lycopersici (*Alexander and Tucker , 1945) and *F.oxysporum.* f. sp. *cubense* in banana (Buddenhagen, 1990). Similarly, the appearance of *Verticillium* wilt was reported, (Alexander, 1962) on previously resistant tomato cultivars and this was further emphasized by Pegg (1974). Nonetheless, Loffler and Rumine (1991) did not find any genetic interaction when they inoculated eight lily

cultivars with eight Dutch and four Italian isolates of *F. oxysporum* f. sp. *lilii ;* all the isolates tested displayed have a similar virulence pattern.

The current study demonstrated the existence of very aggressive isolates of *F.o.e.* notably from Ivory Coast and the New World and this may explain why material selected as resistant against Nigerian isolates has proved susceptible in the Ivory Coast (Renard, , pers. comm. 1991), and material resistant to Zairean isolates was shown to be susceptible to isolates from Brazil (Flood *et al.,* 1993). Isolates from Ivory Coast, Brazil and Ecuador have identical RFLP profiles and are vegetatively compatible (VCG 0141) (Mouya, 1992; J. Flood unpublished). The origin of the out break of Fusarium wilt in Brazil and Ecuador is unclear but seed borne infection is possible (Flood *et al.*, 1994). Those plantations where wilt has occurred received seeds from the Ivory Coast and *F.o.e.* is present on the kernel surfaces of oil palm seeds (Flood *et al.*, 1990).

Isolate Abak 508.1746 was also aggressive and based on the number of inoculated plants that became infected, 16F (Ivory Coast) and Abak (Nigeria) were more aggressive than F_3 from Zaire. Generally, there was very good correlation with Abak and 16F isolates between percentage infection and the severity of symptom development across the fourteen clones tested. However, F_3 induced more severe symptoms in infected palms than Abak. This observation may suggest that the abilities to infect and colonize the host are controlled by different pathogen characteristics. Consequently, Abak is able to infect more plants than F_3 but F_3 has a very high potential to colonize any plant invaded causing more severe symptoms. Thus, F_3 may be able to distinguish between two different resistance mechanisms. For example clone UF28 was resistant to infection but once

infected, was not resistant to vascular colonization. This effect has been observed frequently in this study and by Mepsted (1992).

There have been two contrasting theories postulated on the inheritance of resistance to *F.o.e.* Meunier *et al.* (1979) suggested that resistance was inherited through the action of many genes inherited in an additive manner, whilst de Franqueville and de Greef (1987) proposed that resistance was controlled by the action of only two genes. This latter group conducted their experiments in Zaire and their result may reflect the ability of the Zairean isolates such as F_3 to distinguish between two separate resistance mechanisms.

Oil palm crosses (100% Dumpy) that had been selected for resistance in Zaire using the Zairean isolates did not show external symptoms with any of the aggressive isolates tested. However when the plants were split, vascular necrosis in the bulb was observed particularly with Ivory Coast isolate (16F) which also induced both severe external and internal symptoms in 75% Dumpy. Also many of the other isolates induced internal symptoms and could be reisolated from the Dumpy progenies. Thus, lack of external symptoms in the nursery test and in the field may suggest why these crosses regarded as resistant in Zaire but the high level of aggressiveness of isolate 16F, accounts for the observation that Dumpy crosses were susceptible when tested in the nursery in Ivory Coast (Renard, 1991 pers. comm).

Of the two crosses tested and previously regarded as resistant material in Ivory Coast, cross (b) was confirmed here as resistant, in that very few external symptoms were observed and only isolate 16F, induced severe internal symptoms although several isolates could be reisolated. Nigerian cross (1) exhibited few external symptoms with the majority

of isolates but some isolates did cause disease; 16F was the most aggressive. Cross (2) although reportedly resistant exhibited a wide range of symptoms.

It therefore appeared that oil palm lines regarded as resistant from breeding programmes in Zaire, Ivory Coast and Nigeria, generally exhibited few external symptoms but vascular necrosis was evident and and the fungus could be reisolated. Meunier *et al.* (1979) similarly reported that no oil palm showed immunity and the term tolerant would better describe these host lines.

A comparison of pathogenicity of an isolate of *F. oxysporum* from date palm and oil palm revealed that these isolates were pathogenic on both palm species and this could have important implications for countries such as Sudan, Nigeria where both crops are grown.. However, this preliminary work requires further investigation involving more isolates of both crops and more plant replications before firm conclusions can be reached.

An attempt to investigate if resistance could be expressed *in vitro* was unsuccessful. Both callus lines were colonized equally by *F.o.e.* using either conidial suspension or mycelial blocks and a range of different culture conditions. Holliday and Klarman (1979) demonstrated that the expression of resistance to *Phytophthora megasperma var sojae* in tissue culture was dependent upon levels of 2,4.D in the media. This hormone produced a 'tighter' type of callus and Miller *et al.* (1984) also claimed that concentrations of kinetin on the media influenced alfalfa callus morphology which in turn influenced the expression of resistance to this pathogen. Holliday and Klarman (1979) suggested that a 'tighter' callus may be required for the expression of resistance because a collective response by many cells was needed for resistance to be manifested in tissue culture. However, despite

the manipulation of the media using these hormones, colonization of callus derived from resistant parent line remained similar although colonization of callus from a susceptible line was significantly increased with higher concentrations of 2,4.D.

Similarly,no differential responses were observed in the current study when spore suspensions of *F.o.e.* were applied to callus which is in complete contrast to Kroon *et al.*(1991) studying the interaction between tomato callus and *F oxysporum* f.sp. *lycopersici*. They concluded that the application of spores to callus induced differential responses while inoculation with mycelial blocks masked any response. Also, here, the concentration of spores used had no effect on colonization which again is in complete contrast to findings of Holliday and Klarman (1979) who reported colonization increased with increasing spore concentration.

Thus, it can be concluded from this section that testing *F.oxysporum* isolates from all major oil palm growing regions has shown variations in pathogenicity and aggressiveness but overall no race-cultivar interactions were apparent. Screening for resistance *in vitro* (callus culture) was not successful and consequently alternative strategies for a rapid screening resistance in oil palm are clearly required and are investigated in next Section.

4.0 MECHANISMS OF HOST RESISTANCE AND FUNGAL PATHOGENICITY

4.1 INTRODUCTION.

It is inappropriate to consider one possible mechanism of resistance to vascular wilt fungi in isolation from others and it seems likely that resistance is not dominated by any one factor but is the ultimate expression of several different mechanisms operating in conjunction. However, for convenience, the role of physical and biochemical barriers in resistance will be considered separately in this introduction.

4.1.1. The role of physical barriers in resistance.

A notable feature of vascular wilt diseases is the relative speed with which the fungus is able to move up the plant and also the containment of the pathogen within the vascular system of the host throughout the major period of pathogenesis (Beckman *et al.,* 1976; Beckman and Talboys, 1981; Cooper, 1981). The distance moved by fungal propagules varies from a few centimetres in a young root to several metres in a mature plant depending on the vessel lengths of the species involved. It is mainly at the vessel end walls (or trap sites) where the propagules germinate and may grow through or past the obstruction, forming a new generation of propagules which then repeat the sequence distributing the pathogen throughout the plant. Lateral growth through pits into adjacent vessels also occurs, but the extent to which this is possible may also be limited by the distribution of xylem parenchyma and ray cells which are not invaded at this stage of pathogenesis (Talboys, 1972).

Thus, the innate structure of the xylem elements (vessel lengths, diameter branching and contiguity) may influence the ability of certain plant species to localise vascular infections.

Vascular anatomy, in particular, vessel lengths may have a role resistance in relation to the rate of upward distribution of propagules in the vascular system. In a study conducted on elms resistant and susceptible to Dutch elm disease (DED), Banfield (1968) related resistance to xylem anatomy. He observed that the short period of susceptibility in resistant clone correlated well with early formation of wood and ceased with initiation of late wood which in turn was characterised by a lack of interconnections between individual vessels and vascular bundles. He concluded that the resistance expressed by late wood depended on a restriction of spore movement due to the lack of vessel articulation. Similarly, Elgersma (1970) suggested that there was a fundamental difference in xylem structure between resistant and susceptible elms which was based on the slower flow of air, dye and water through DED resistant clones. In a detailed study of elm xylem structure involving many clones varying in susceptibility to DED, a positive correlation was established between vessel group size (product of mean number of contiguous vessels and vessel diameter) and susceptibility (McNabb *et al.,* 1970). In their conclusion, they suggested that since lateral distribution of the pathogen was through bordered pits there were fewer points of contact with other vessels which could have contributed to resistance.

In contrast to the above studies, most results obtained from investigations conducted on various species of herbaceous plants have shown no direct link between resistance to fungi and vessel diameter, length or articulation (Beckman *et al.,* 1976; Elgersma *et al.*, 1972; Mace *et al.,* 1971; Presley *et al.,* 1966; Presley and Taylor, 1969). Thus, initial transport was unaffected by morphological features of stem xylem in tomato isolines, susceptible and resistant to Fusarium wilt (Elgersma *et al.,* 1972; Mace *et a.,* 1971). However, in some instances vascular anatomy may be involved in resistance of some herbaceous plant

species to bacterial diseases. Cho *et al.* (1973) attributed the resistance of alfalfa to bacterial wilt (*Corynebacterium insidiosum)* as being due to shorter xylem vessels. Similarly, a low number of large contiguous vessels was proposed as a factor in resistance to Ratoon Stunt Disease of sugar cane caused by a small unidentified bacterium (Teakle *et al.,* 1978). In this investigation, the rate of water flow sucked through healthy, single node cuttings of sugar cane clones was slower in the resistant clones (more profuse branching of the large metaxylem vessels in the nodes) than susceptible clones.

There has been no comparable study of the vascular system of oil palm but in studies of palm vascular anatomy, Zimmermann and Sperry (1983) and Sperry (1986), found that in the palm (*Rhapis excelsa)* vascular flow in the trunk occurs in long, wide diameter, xylem vessels of low resistance. They also observed constriction in the leaf bases caused by small metaxylem vessels.

Another expression of resistance is the physical localisation of the parasite by occlusion of the vessels with gels and/or tyloses. It has been hypothesised that such mechanisms may explain the differential responses of resistant and susceptible cultivars /clones of various species to vascular pathogens (Beckman, 1964; 1966; Beckman *et al.*, 1972; Elgersma, 1973; Elgersma *et al.,* 1972; Hutson and Smith, 1980; Shi *et al.,* 1992).

The observation that gels extend from the upper surface of perforation plates and end walls in infected vessels suggest that they result from a considerable swelling of the pit membrane which become distended into the upper vessel by the transpiration pull (Beckman, 1964). This observation was further reinforced when Beckman (1969 a) induced the swelling of artificial membranes by exposure to alternating high and low pH. He suggested that such a fluctuation in pH could occur on a diurnal basis in infected plants as a result of a build up of respiratory carbon dioxide at night (Beckman, 1967; 1969b).

There have been reports of gel production as a result of polygalacturonase and ethylene action (Van der Molen *et al.,* 1983) and in response to various stresses (Burdett, 1970). Tylosis (outgrowth of vascular parenchyma cells) is also a non-specific response that occurs in a wide variety of plants and can be induced by infection, wounding or various stresses. Tyloses may occur in the roots, primary xylem and the first secondary xylem of the lower part of the stem even before symptom development (Talboys, 1958; Beckman *et al.,* 1972). The xylem parenchyma cell walls including the protective layer become elasticised and balloon out through the pit pairs into the vessel lumen (Beckman, 1971). The cellulosic walls of the tyloses expand to such a degree, and they may form in such numbers, that the xylem vessels may become completely blocked. Although this restricts water transport through these vessels, vascular colonisation by pathogens is retarded. Indeed, both gels and tyloses exhibit the capability of physical localisation of the parasite by occlusion of the vessels. In a study conducted to detect differences in build up and distribution of *Verticillium dahliae* and *F. oxysporum* f. sp. *vasinfectum* within the vascular tissues of wilt resistant and susceptible cotton cultivars, Harrison and Beckman (1982) found that the build up of both pathogens in a susceptible cultivar one to two days after tap root inoculation, was both extensive and intensive. This was evident when they measured propagules per tap root segment, number of infected xylem vessels and propagules per infected vessel. In contrast, colonisation of the resistant cultivar was restricted to the zone where inoculum was introduced; resistance correlated with production of gels and tyloses. Previous detailed study of the disease process in *Fusarium*-infected banana had led Beckman (1964) to hypothesise a convincing role for gels and tyloses in host resistance. He had postulated that following the initial penetration into the vascular system, the fungus sporulates and the spores are carried up to the first trapping

site. Three days are required for penetration of the pit membrane and production of second generation of spores but during these 3 days gels are formed which immobilise the pathogen. In the resistant reaction, tyloses form after three to six days, approximately 5 - 8 cm from the trapping site and permanently seal off the vessels. Gels produced in the susceptible reaction become weakened and break, allowing further colonisation. Tylosis is delayed and thus develops too late to prevent passage of the pathogen. He attributed the non persistence of gels on susceptible reactions to one or more factors. For example, the amount of hemicellulose B in banana cell walls has been correlated with resistance to *F. oxysporum.* f. sp. *cubense.* Calcium pectate and pectin are the major components of gels but hemicellulose B may confer durability on the gels by protecting them from the action of pectolytic enzymes (Zaroogian and Beckman, 1968). Calcium pectate is resistant to PG activity and hence a greater degree of cross linking by $Ca^{2}+$ in resistant gels would give added strength (Bateman, 1964; Cooper,1974). The infusion of phenolics into gels occurs four to six days after infection in banana roots and also confers resistance to enzymic degradation (Beckman *et al.*, 1974). A delay in phenolic infusion could enable the pathogen to break through the gel.

Subsequently, the role of gels and tyloses in resistance/susceptibility has been reported in other host pathogen interactions including hop, tomato and cotton inoculated with *Verticillium* where tylose formation occurred ahead of fungal colonisation (Mace, 1978; Sinha and Wood, 1967). In all these interactions an inverse relationship occurred between Tyloses and hyphal colonisation while in contrast, in studies of mechanisms of resistance in *Verticillium* infected hop roots (Talboys, 1958) and in tomato plants (Blackhurst and Wood, 1963) the time of appearance of tyloses relative to hyphal colonisation did not support the view that they represent primary resistance mechanism in the tested plants.

4.1.2. The role of biochemical barriers in resistance.

In addition to morphological barriers, chemical compounds have also been implicated in resistance. Plants possess a wide range of substances some of which exhibit antimicrobial properties. For example, saponins (steroid molecules) have been implicated as preformed resistant determinants in many food crops. Recently, Osbourn, *et al.*(1994) cloned the gene which encode the enzyme, avenacinase, which detoxifies the oat root saponin avenacin A - 1, from the oat infecting fungus, *Gaeumannomyces graminis* var *avenae.* Avenacinase - minus mutants of this fungus were unable to infect oats but were pathogenic to non-saponin - containing host, wheat. These workers also demonstrated similar genetic evidence for tomato saponin ,tomatine, from tomato fungi, *Septoria lycopersici, V. albo atrum* and *F. oxysporum* f. sp. *lycopersici.* Another example of a preformed antimicrobial compound is catechol in resistant onion bulb scales which inhibit spore germination of *Colletotricum circinans,* the causal agent of onion smudge disease (Link *et al.* 1929).

In addition to constitutive substances, induced toxic compounds have been linked with resistance in many plant species. These, phytoalexins, are defined as low molecular weight antimicrobial compounds that are both synthesised by and accumulate in plants which have been exposed to micro-organisms or injury and are synthesised from remote precursors, probably through *de novo* synthesis of enzymes (Mansfield and Bailey, 1982). Many different phytoalexins have been isolated and characterised from a range of plant families, for example, Leguminoseae, Solanaceae, Orchidaceae and Gramineae (Dixon, 1986). The compounds are diverse and belong to a number of chemical groups including flavonoids

(pisatin and phaseollin), coumarins, polyacetylenes (wyerone), terpenoids (rishitin and gossypol) and steroids.

Bailey (1982) proposed that phytoalexin biosynthesis is activated by endogenous elicitors released from cells undergoing the hypersensitive reaction of dying as a result of exposure to various phytotoxic substances. An alternative to cell death has also been suggested which involves recognition where direct and specific elicitation of phytoalexin biosynthesis may occur by elicitors of microbial origin (Bruegger and Keen, 1979; Keen and Legrand, 1980). In a study on mode of biochemical action of phytoalexins, Yoshikawa *et al.* (1987) claimed that the plasma membrane of the pathogen is rapidly disrupted and the structural integrity is affected resulting in massive loss of electrolytes leading also to disruption of respiratory path ways.

Most reports supporting a role for phytoalexin accumulation as the cause of inhibition of microbial growth in resistant plants come from interactions in which resistance is expressed following penetration and is associated with the necrosis of plant cells (Collinge *et al.*, 1987). For example, Bailey and Deverall (1971) recovered more than 150 mg/g fresh weight phaseollin from the incompatible interactions between *Colletotrichum lindemuthianum* and French bean hypocotyls; this concentration being about 300 times that required for inhibition of spore germination. However, they were unable to detect phaseollin during the biotrophic period of colonization by a compatible race. Some accumulation of this phytoalexin was recovered after the death of infected tissues and formation of lesions but the concentrations were low (5 mg/g). Similarly, in studies on broad bean infected with necrotrophic pathogen, *Botrytis fabae* (causal agent of Chocolate spot in broad bean) and the pathogenic *B. cinerea,* Hargreaves *et al.* (1977) were able to extract wyerone derivatives from the incompatible interaction. These workers also

examined spreading lesions of the compatible interaction and observed that an initial increase in phytoalexin concentration within the mixture of live and dead cells at inoculation sites was followed by a decrease as tissues became completely necrotic and colonized by

B. fabae. Their results suggested that phytoalexin accumulation restricts the growth of *B. cinerea* and that *B. fabae* is able to metabolize and detoxify the inhibitors to which it is exposed and thereby to prevent their accumulation to fungitoxic concentrations around invading hyphae.

Studies on the accumulation of phytoalexins and their effects on fungal growth have also been conducted on vascular wilt diseases of plants that include *Verticillium*-infected tomato (rishitin) (Tjamos and Smith, (1974); Hutson and Smith, (1980), lucerne (medicarpin) (Flood *et al.,* 1978; Flood and Milton, 1982) and cotton (sesquiterpenoid) (Garas and Waiss, 1986) and also from *Fusarium* infected pigeon pea (isoflavonoid) (Marley and Hillocks, 1993), tomato (rishitin) (Harrison and Beckman, 1987) and cotton (terpenoid) (Shi and Beckman, 1992; Zhang *et al.,* 1993). Less information is available on phytoalexins in palms although compound(s) have been extracted from resistant (but not susceptible) date palms which were inhibitory to *F. oxysporum* f. sp. *albedinis* (Bayoud disease) (Assef *et al.*,1986). Similarly accumulation of antifungal compounds have been reported from the roots of *F.o.e.* infected resistant oil palms (Taquet *et al.,* 1985); Vernenghi *et al.*, 1987 and Diabate *et al.*,1990).These substances were claimed to be of phenolic nature particularly benzoic and cinnamic acids derivatives, but no detailed studies of their chemical composition was made. These authors reported their accumulation in the tissues (roots and pseudobulbs) following infection and at different concentrations depending on the genetic origins of the progenies tested, and demonstrated that the

compounds were inhibitory *in vitro* to spore germination but their role in resistance was not established.

The presence of phytoalexins in the vascular system in combination with other resistance structures (eg. gels and tyloses) maybe significant, as gels and tyloses slow down vascular flow which thus allows phytoalexins to accumulate and subsequently prevent further colonization. Therefore, based on studies on *Verticillium* infected cotton, Mace (1978) concluded that in the incompatible interaction vessels occluded with gels and tyloses and the trapped fungal hyphae and conidia are exposed to fungitoxic concentrations. However, in the compatible interaction, vessel occlusion is delayed thus allowing conidia to escape entrapment and consequent exposure to toxic levels of the phytoalexin(s).

Other chemicals which may have a role as resistance or pathogenicity factors are enzymes which may have antimicrobial activity or result in antimicrobial products, thus disease resistance may relate to increased levels of activity and the speed of synthesis of these enzymes.

Browning of colonized xylem vessels and the surrounding parenchymatous tissues has long been described as one of the characteristic internal symptoms of wilt diseases (Maraite, 1973). Polyphenoloxidase (PPO) and peroxidase (PO) oxidize phenols to more fungi toxic quinone derivatives and PO appears to be associated with resistance by catalyzing the final step in the polymerization of lignin synthesis (Hammerschmidt and Kuc, 1982). Thus, rapid changes in the activities of these enzymes after infection of resistant plants, may indicate their possible role in resistance responses (Retig, 1974). This author observed that PPO and PO activity increased significantly in roots of a resistant cultivar of tomato within 24 h of inoculation with *F. oxysporum* f. sp. *lycopersici* whilst in a susceptible cultivar, PO activity did not show a similar increase until 24 h later and no

increase in PPO activity was detected. In contrast, recently Mozzetti *et al.* (1995) found higher PO activity only in uninoculated resistant pepper plants and they associated the variations they observed in these enzyme activities in *Phytophthora* inoculated plants with severe disease rather than resistance. One of the sources of substrate for the phenoloxidase may arise from the synthesis of aromatic compounds from transcinnamic acid, the immediate product of phenylalanine ammonia-lyase (PAL) reaction (Ebel *et al.,* 1984).

PAL is a key biosynthetic enzyme at the head of phenolic biosynthetic pathway, some of which are required for the biosynthesis of lignin and other for constitutive or induced antimicrobials. Thus, rapid increase in PAL mRNA transcription and in PAL activity have received much attention in many host pathogen interactions (Ebel *et al,* 1984; Hahlbrock and Scheel, 1989). In resistant *Eucalyptus* roots inoculated with *Phytophthora cinnamoni,* Cahill and McComb (1992) found an increase in PAL activity within 24 h of inoculation. However, Mozzetti *et al,* (1995) were unable to detect PAL activity in *Phytophthora*-pepper interactions.

Other compounds linked with resistance may also be elicited by invading pathogens. Fungal cell walls generally contain chitin and /or glucan, therefore the corresponding host depolymerases,chitinases and glucanases are potential inhibitors of fungal growth (Hadwiger and Loschke, 1981; Schlumbaum *et al.,* 1986).

4.1.3.<u>The role of biochemical factors in pathogenicity</u>.

Much attention has been drawn to a wide range of pathogenicity factors for example, cell wall degrading enzymes (CWDE) and toxins all of which have been implicated in vascular wilt diseases but evidence of their role as pathogenicity determinants remains equivocal. In

this current study consideration will only be given to CWDE because their activity could cause disruption to vascular pectic gels and their ability to macerate host tissue.

Phytopathogenic microorganisms produce an array of enzymes of which pectinases are one of many groups which are considered as putative pathogenicity determinants /factors of vascular fungi (Cooper, 1983). Vascular wilt fungi transverse xylem walls via the fragile primary walls of pit membranes. These structures constitute a group of complex acidic polysaccharides which occur in varying amounts in plant materials; pectic polysaccharides are important gelling agents in vascular wilt diseases (Cooper, 1983). The composition of vascular gels and proportions of carbohydrate polymers are similar to those of primary cell walls (Beckman and Zaroogian, 1967; Vander Molen *et al.,* 1982). These pectic polymers are polygalacturonides with other non-uronides bound to the unbranched chain of α-1, 4-linked Gal A interpersed with 1, 2-link Rha=rhamnogalacturonan). Thus, a successful vascular pathogen must hydrolyse structures within 2-3 d after infection to avoid triggering other resistance factors or exposure to,for example, tyloses, phenolics and phytoalexins. Endo-polygalacturonase (endo-PG) and endo-pectinlyase (endo-PL) have been implicated as enzymes which can degrade vascular gels and therefore have been associated with pathogenicity (Beckman, 1964). This author also reported the degradation of gels in banana plants infected with *F. oxysporum* f. sp.*cubense.* Cooper and Wood (1980) observed vessels containing gels and pit membranes which had been degraded by endo-PG in tomato roots inoculated with *V. albo-atrum.* Also, a transmission electron microscopy study on susceptible tomato infected with *F. oxysporum* f. sp. *lycopersici* or *V. albo-atrum* and of pea, infected with *F. oxysporum* f. sp. *pisi,* Bishop and Cooper (1983) showed extensive degradation of primary wall and pit membranes by CWDE.

In addition, pectic enzymes can kill host cells; Bateman (1976) suggested that tissue degradation by endo-pectinases may result in cell separation or maceration so that it is no longer able to support the protoplast which is under osmotic stress and invariably accompanied by cell death. However, the mechanism(s) by which plasma membranes are damaged in the presence of these enzymes remains unclear. This view of cell death resulting from membrane disruption was originally proposed by Hall an d Wood (1973). Although this view appears to be widely accepted, some reports have given evidence of rapid effects of endo-PL on the plasmalemma before the cell wall is apparently altered (Hislop *et al.,* 1979).

Detection of pectic enzymes from infected plant tissue provides a good basis for establishing a role in pathogenesis. However, an important aspect to consider in studying the involvement of CWDE in pathogenesis is whether an increase in pectinase activity precedes or occurs concurrently with symptom formation as CWDE may be irrelevant to pathogenesis and may be produced during a period of saprotrophy. Cooper and Wood (1980) found that maximum activity of these enzymes preceded symptom expression in susceptible tomato cuttings 3 d after inoculation with *V. albo-atrum.* Also, in *Verticillium* infected lucerne, PL levels were found to increase simultaneously with the onset of wilting (Heale and Gupta, 1972) and PL activity was detected in susceptible onion bulbs at the onset of rot symptoms caused by *F. oxysporum* f. sp. *cepae* (Holtz and Knox-Davis, 1985 b).

Although many workers have suggested a possible contribution of pectic enzymes to the aggressiveness of vascular wilt fungi, consistent correlations have been lacking (Artes and Tena, 1990; Osagie and Obuekwe, 1991; Fernandes *et al.,* 1993). Since enzyme production is regulated by a system of induction and repression, it would appear that the

demonstration of CWDE *in vitro* under one set of conditions is not a guarantee that they will be produced in the same quantity or at all *in vivo* (Cooper and Wood, 1973; 1975). The use of enzyme deficient mutants is perhaps a more promising line of approach in determining the role of pectic enzymes as pathogenicity determinants (Puhalla and Howell, 1975; Cooper, 1983). Puhalla and Howell (1975) were able to induce firstly PG- and later PL- deficient mutants of *V. dahliae* but the mutants retained their pathogenicity for cotton thus casting some doubt on the role of these enzymes (Howell, 1976). Similarly, the pathogenicity of three PL deficient mutants of *V. albo-atrum* was compared to the wild type in tomato plants (Durrands and Cooper, 1988). These workers showed these mutants could colonize the host in the absence of symptoms and concluded that pectinases are virulence factors rather than pathogenicity determinants.

The aims of this section were to investigate:

1. To test if resistance could be expressed in oil palm roots or petioles.

2. To investigate the basis of this resistance, for example, antifungal substances, morphological barriers and host produced enzymes.

3. To develop a model system for rapid screening for resistance based on parts 1 and 2.

4.2 RESULTS.

4.2.1 The expression of resistance in cut roots *in situ*

4.2.1.1 Fungal colonization in roots of four oil palm clones of varying degrees of resistance and susceptibility

The extent of colonization of oil palm roots by *F.o.e.* was investigated in four clones of varying levels of resistance in order to provide a tissue in which pathogen and host reaction could be investigated in a defined space and time period. The plants selected for this experiment had previously been screened for resistance in the glass house (Section 3) and their wilt reaction was known.

Mature roots still attached to the plants (1-2 years old) were severed under tap water at a distance of 10 cm from the pseudobulb. One suitable root on each of five replicate plants per clone was treated per experiment (2.4). Inoculation was conducted as before with a standard isolate (F_3) and fluorescent tracer particles added to conidia to locate possible sites of host response at trapping sites such as vessel end walls (2.10). These particles could easily be detected under UV light (Beckman *et al.*, 1962). After 14 d incubation, the roots were removed from the plant, rinsed under tap water and free hand sections were cut at 0.5 cm intervals along 5 cm length of each root from the point of inoculation. These sections were stained and examined as before (2.9.1).

Trap sites were easily identified by the presence of fluorescent particles in the vessels (Plate 2a) and the vessels colonized by hypae were visible (Plate 2b) (Table 11). Secondary colonization in the adjacent or lateral vessels was absent or occurred so infrequently that quantification was not attempted.

Table 11: Colonisation of four clonal oil palm lines after inoculation with *F. oxysporum* f. sp. *elaeidis*.

CLONE	WILT REACTION	NUMBER OF TRAPSITES WITH:WITHOUT HYPHAE
UF28	Resistant	15:83 a
UF136	Moderately resistant	17:41 b
UF174	Susceptible	34:52 b
UF177	Highly susceptible	46:37 c

Values with the same letter are nsd (p=>0.05) using X^2 analysis as determined previously (Section 3)

Colonization was significantly higher in the highly susceptible clone 4 compared to the other clones especially the resistant clone 1. There appeared to be more colonization in the susceptible clone than in the moderately resistant clone but this was not significant. Nevertheless, there was a trend that colonization at 'trap sites' correlated with wilt reaction at the whole plant level.

Plate 2a: Trapsite in root xylem vessel identified by the presence of fluorescent particles. (Bar marker represents 50 μm).

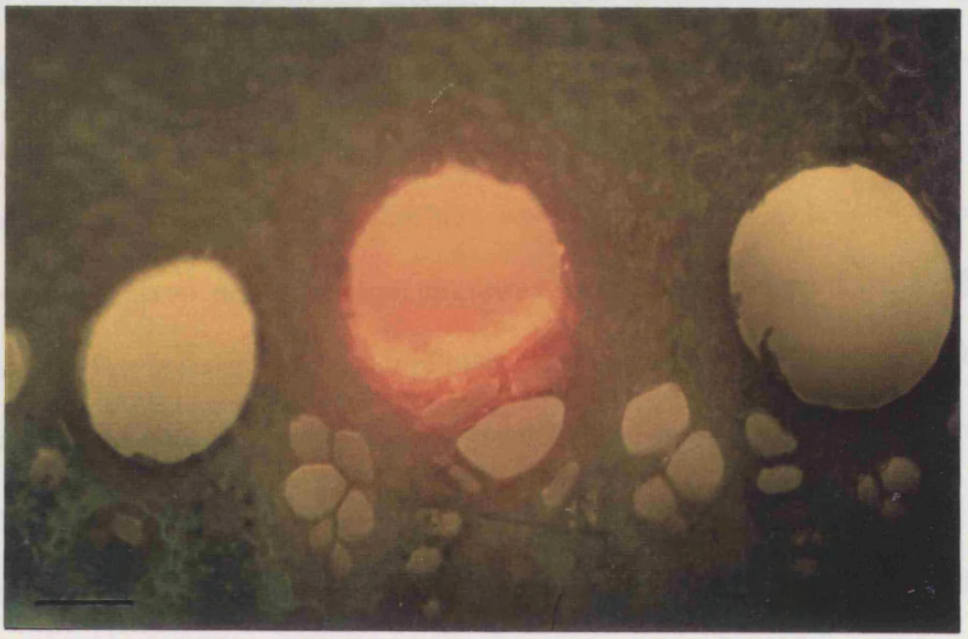
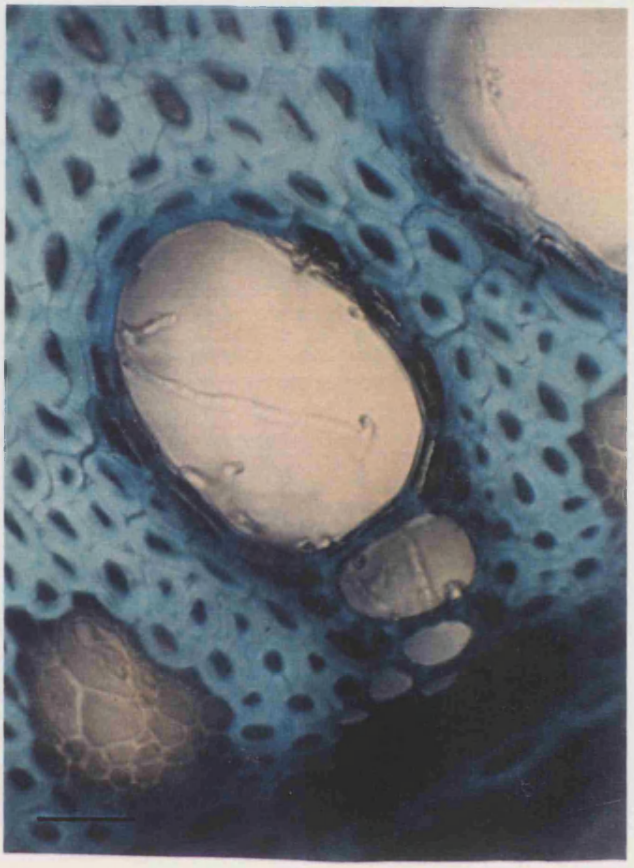


Plate 2b: Colonization of xylem vessels by *F.o.e.* (Bar marker represents 50 μm).

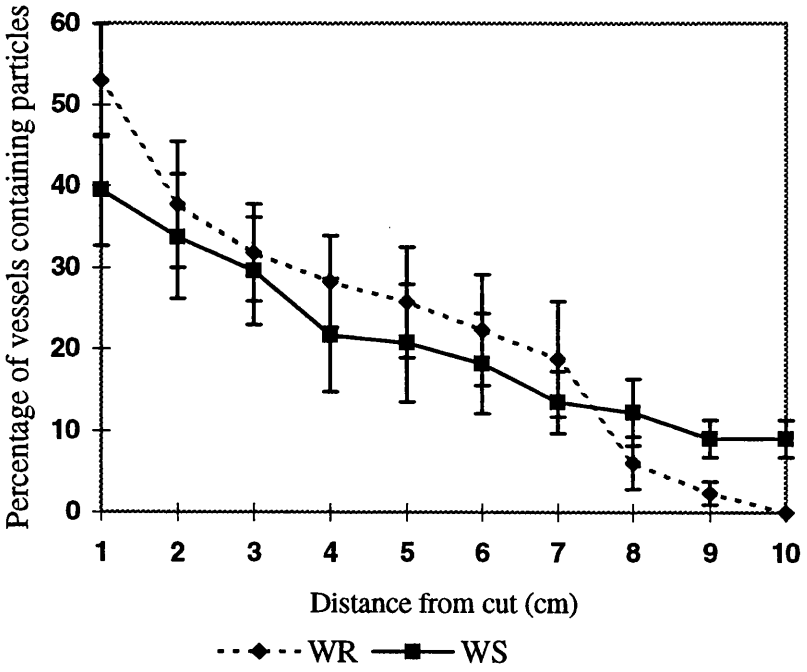


4.2.1.2 Preformed physical barriers in roots; determination of vessel lengths.

Previous results showed significantly higher fungal colonization in vessels of susceptible plants than in resistant. Initial distance travelled by spores during natural transpiration in xylem fluid following inoculation depends on vessel lengths of the host (Beckman *et al.*,1961). Thus, an experiment was conducted to investigate if host resistance to vascular colonization could be related to vessel lengths.

Palms of extreme resistance or susceptibility were chosen and one suitable root on each of five replicate plants per clone was inoculated with fluorescent particles as described previously. This revealed the number of functional vessels in each of the three sections cut per interval along the segment up to approximately 3 cm below pseudobulb. Vessel lengths were determined as previously described (2.10).

Figure 2 Vessel lengths in roots of (WR) and (WS) clones revealed by fluorescent particles.



There was no significant difference in vessel lengths between resistant and susceptible clones; *ca.*40 and 54% of vessels in susceptible and resistant clones respectively contained fluorescent particles 1 cm from cut end (Fig.2). The number of vessels containing fluorescent particles gradually declined in both clones with distance from the cut end and at 8 cm (i.e. 2 cm below pseudobulb) there appeared to be a sharp reduction in the number of vessels containing fluorescent particles (*ca.* 10%) in the resistant compared to the susceptible clone (*ca.*18%) but again this was not significantly difference (P>0.05 Mann Whitney test). Additional sectioning beyond 10 cm revealed presence of fluorescent particles extending to the root pseudobulb junction indicating that some vessels extended the entire length of the root in the susceptible clone.

4.2.1.3 Quantification of *F.o.e.* and in relation to inhibitors in resistant and susceptible oil palm roots.

It had been observed in a previous study that more colonization by *F.o.e.* occurred in the susceptible clone than in the resistant clone after 3h inoculation by natural transpiration. This experiment was planned to investigate if preformed inhibitory compound(s) could explain the reduction of the amount of *F.o.e.*. recovered in the WR clone.

Five replicate mature roots (one per plant) from a resistant and a susceptible oil palm clone were chosen and excised to *ca.* 5 cm in length. These segments were surface sterilized for 10 minutes in 10% (v/v) sodium hypochlorite containing a drop of Tween 80, then washed twice in SDW. Each root segment was thinly sliced with a sterile razor blade and the slices combined and comminuted with a pestle and mortar containing 10 ml of SDW and 5 ml of acid-washed sand. The homogenate was divided into two aliquots. From one aliquot folowing addition of 1 ml of spore suspention (3×10^7 spore ml^{-1}), 1 ml samples from a dilution series in SDW were immediately plated onto *Fusarium* selective medium

(Papavizas, 1967) augmented with antibiotics (Appendix 1). To the other aliquot, 1 ml of spore suspension (3 x 10^7 spores ml^{-1}) was incorporated into the aliquot and allowed to stand for 3 h at room temperature to mimic inoculation conditions by exposing conidia to potential inhibitors in the aliquots before extraction and assessment for viable *F.o.e.* propagules. After 3 h incubation, dilutions series were made and plated as before. All treatments were incubated for 4d at 28°C and the amount of viable fungus (as cfu) present was determined.

Table 12: Quantitative assessment of viable fungus in homogenates of resistant and susceptible palm roots

WILT REACTION	TIME OF SPORE EXPOSURE TO ROOT HOMOGENATE (h)	COLONY FORMING UNITS (cfus) x10^3
WR	0	38.3 a
	3	10.8 b
WS	0	56.3 a
	3	14.8 b

Values represent means of five replicates.

Within each column values with the same letter are nsd (P>0.05) using Kruskal Wallis and Mann Whitney repeated tests.

There were no significant differences between clones in the number of colonies in root homogenates whether at time zero or after 3h incubation (Table 12). However, there were significant differences between counts taken at time zero and 3 h within each clone. This

suggested the gradual release, activation or action of fungitoxic factors in both clones. Extraction for antifungal compoounds in roots and petioles is described later in this section.

4.2.1.4 Induced physical barriers in roots;temporal accumulation of gels, tyloses and fungal growth in xylem vessels of resistant and susceptible roots.

Resistance to vascular wilt pathogens may be expressed by the early production and accumulation of physical barriers to exclude the pathogen from infecting adjacent or subsequent xylem vessels (Beckman *et al.,* 1992). To investigate this potential, five roots from wilt resistant (WR) and wilt susceptible (WS) palms (approx. 5 cm below the bulb) were inoculated as above. Gel accumulation, production of tyloses and fungal colonization were assessed 4 and 8 d after inoculation using freehand sections cut at 0.5 cm intervals along each root and viewed microscopically.

Gels were produced within 4d of inoculation while tyloses were not observed until 8d post inoculation. Gels accumulated to significantly higher levels in WR than in WS plants 4d and 8 d post inoculation (Figs. 3b and 3d). Four days after inoculation, the highest colonization of WR clone occurred 1cm from the point of inoculation (Fig 3a) while the greatest intensity of gel accumulation occurred 1cm above this (Fig 3b). The WS clone showed little colonization and gel accumulation (Figs.3a and 3b). However, by 8 days, colonization in the WS clone had increased (40% vessels occluded at 1cm from cut end) and was significantly higher than in the WR clone (Fig 3c). Gel accumulation had increased in both clones but was significantly higher in WR clone (Fig 3d).

Figure3a: Colonization of roots 4 days after inoculation.

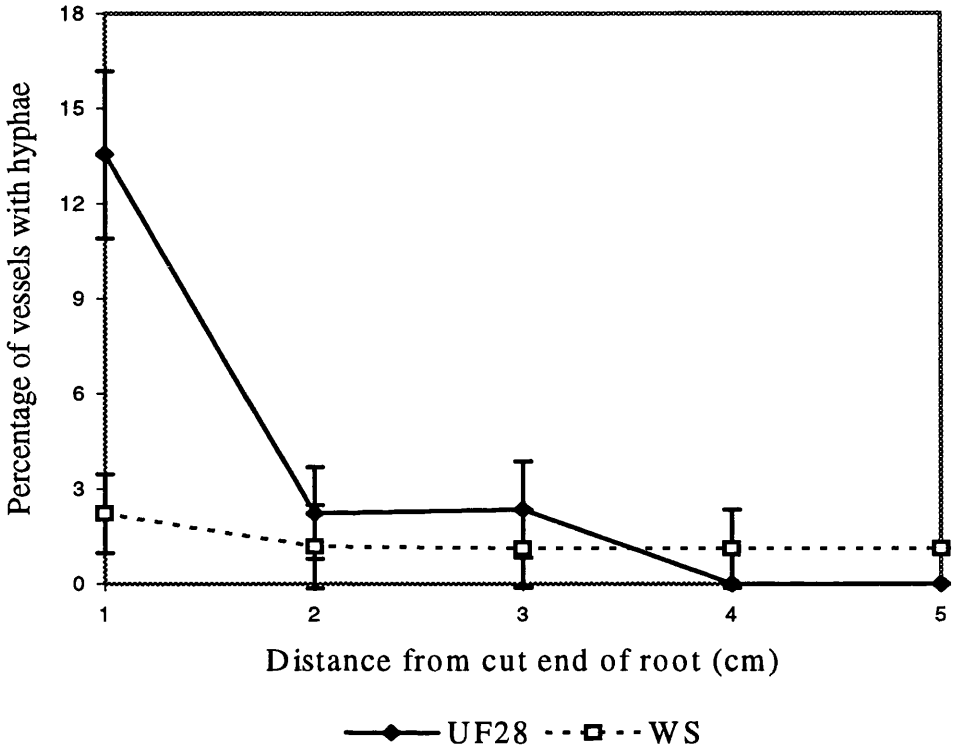


Figure 3b: Accumulation of gels in roots 4d after inoculation.

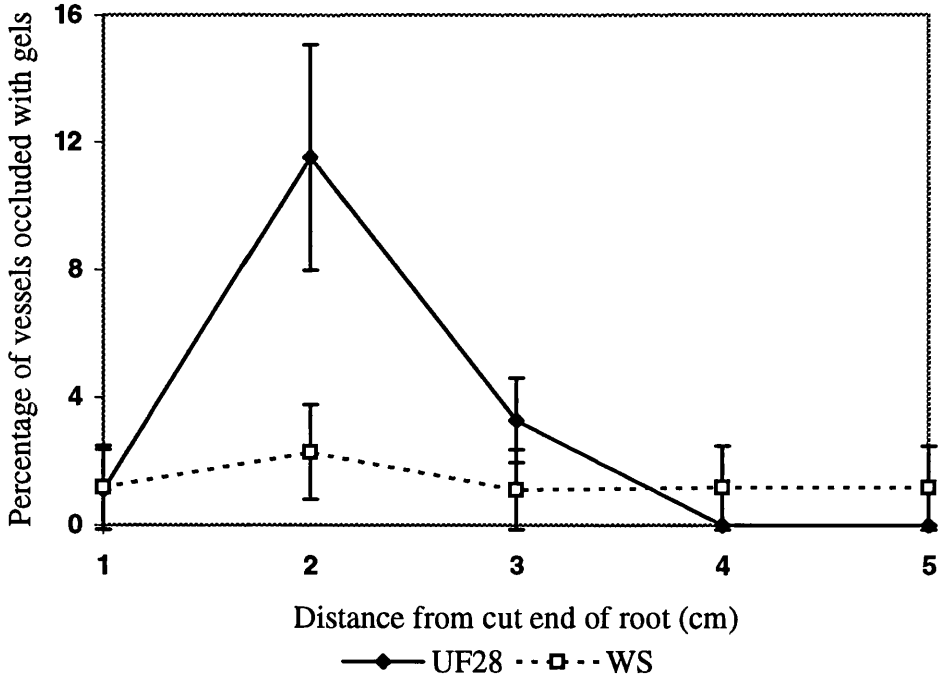


Figure3c: Colonization of roots 8 days after inoculation.

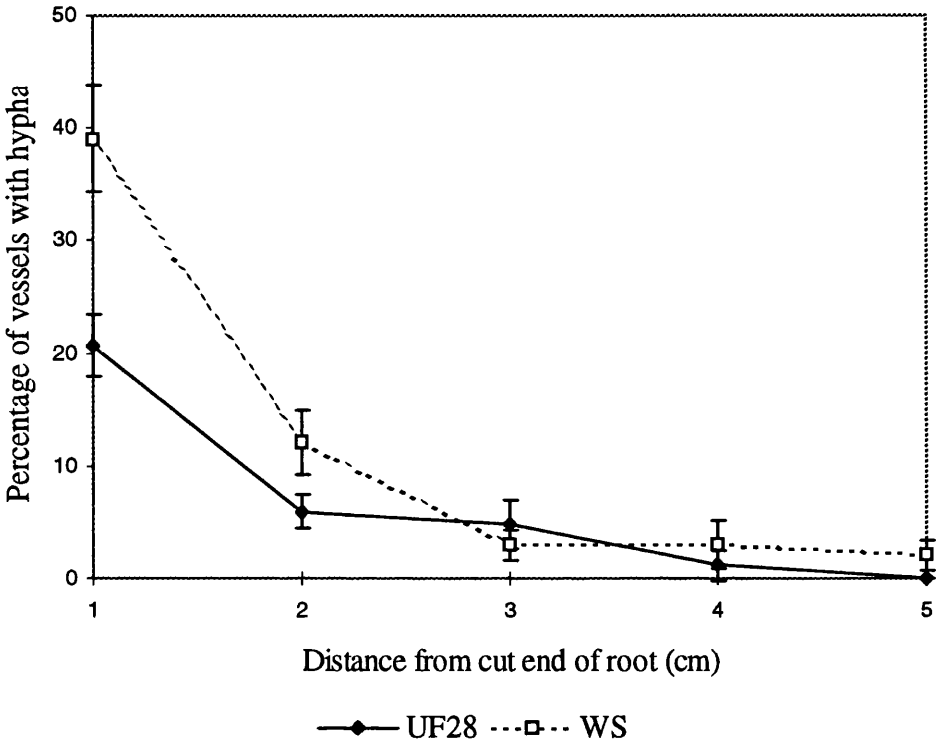


Figure 3d:Accumulation of gels in roots 8d after inoculation.

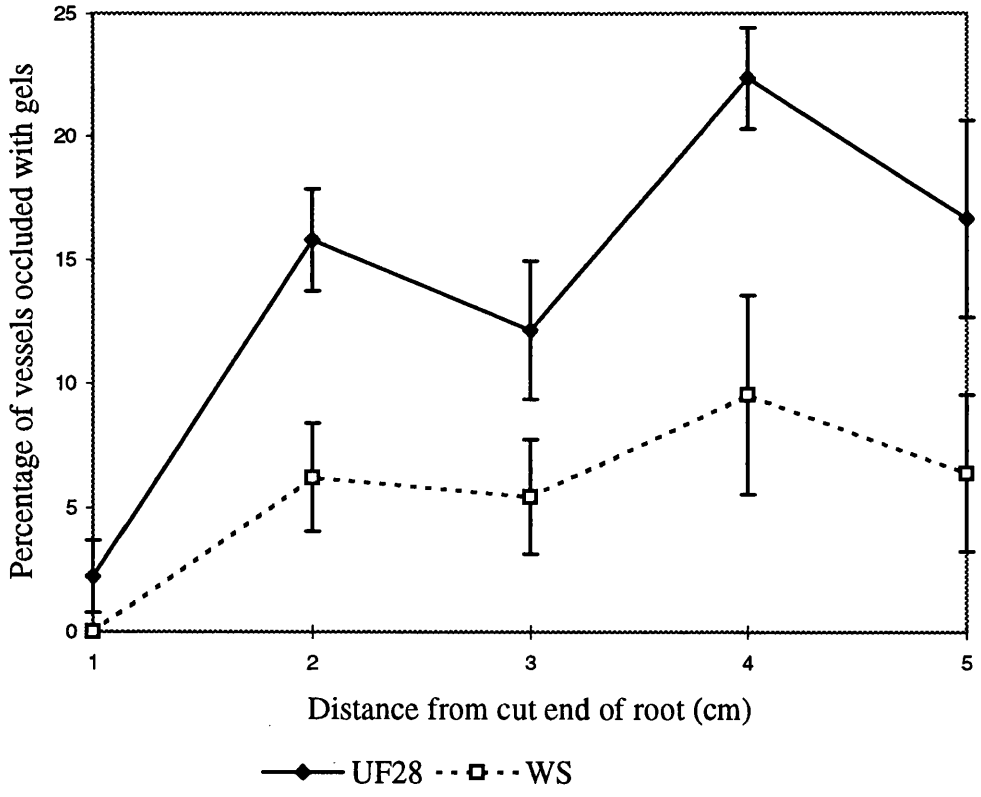
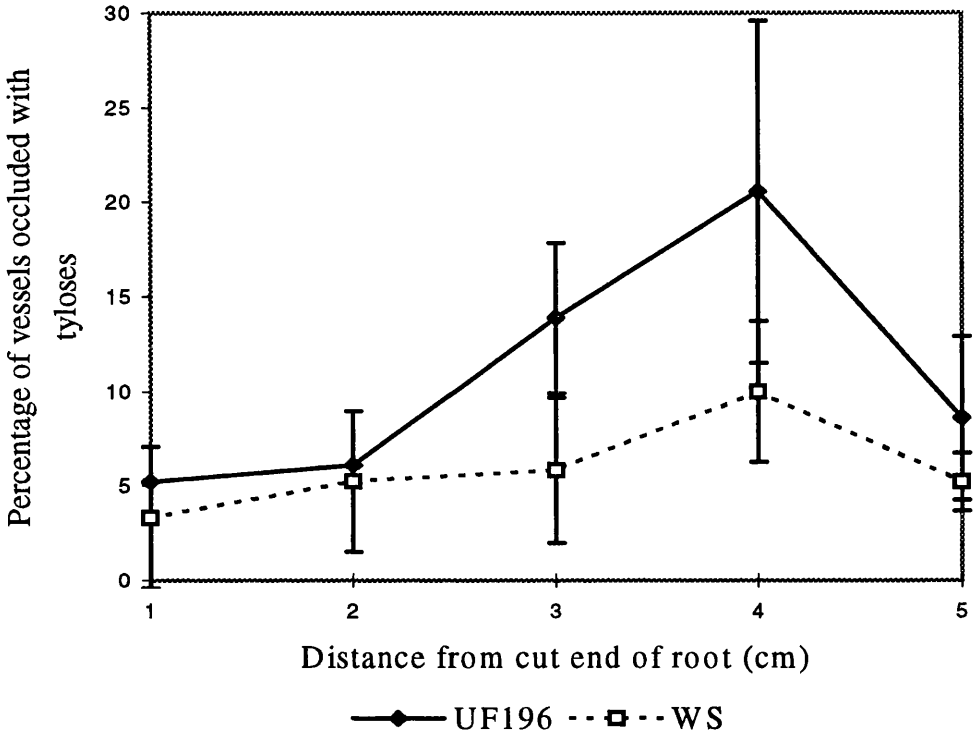


Figure 3e. :Accumulation of tyloses in roots 8d after inoculation.



Vessel occlusion with tyloses was higher in the WR clone although this was not significant from the WS Clone. In both clones, vessel occlusion was greatest 4 cm from the point of inoculation (22% in WR clone and 11% in WS clone) (Fig.3e).

Some gelation was also observed in wound controls in both clones.

In summary, colonization of xylem of cut roots by *F.o.e.* was significantly lower in a highly resistant clone compared to the susceptible clones. However, no significant difference in colonization in moderately resistant and susceptible clones was evident by this technique. Most xylem vessel lengths in oil palm roots were less than than 1 cm, a few (5%) were up to 9 cm but none extended into the pseudobulb in the resistant clone. However, in the susceptible clone 15% of vessels were longer than 10 cm and thus extended into the pseudobulb.

Vascular occlusion was induced in several wound inoculated roots in both clones. Fungal colonization in WR clone was restricted by the more rapid occlusion of vessels with gels and tyloses above the area of colonization. In contrast, colonization in the susceptible clone was significantly higher and associated with lower and delayed production of gels and tyloses in the vessels.

4.2.2 Development of a model system for rapid screening for resistance in oil palm petioles

4.2.2.1 Symptom expression and fungal colonization of infected petioles from WR and WS clones.

Preliminary investigations to study expression of resistance and susceptibility 4 and 8 d after inoculation were conducted on severed intact oil palm roots. Although resistance was expressed in roots,the use of intact roots in this study was abandoned because of the destruction of limited plant material and the difficulty of handling the material experimentally. Consequently, alternative material was sought and it was decided to investigate expression of resistance and susceptibility in oil palm petioles. Petioles from youngest ,mature leaves from 10 plants of WS and WR clones were severed under tap water (2 petioles per plant) and placed upright in an Eppendorf containing 0.5 ml of spore suspension (5 x 10^6 spores ml^{-1}). After 3 h of natural transpiration in the glass house, each petiole was transferred singly into a 150 ml flask containing 100 ml of sterile distilled water (SDW) and allowed to transpire as described previously. Eight days after inoculation, leaves were excised from the petioles and 5 cm long segments cut from each petiole from the site of inoculation. The petioles were immediately scored for visual external symptoms. Petioles were then preserved in FAA and fungal colonization assessed microscopically by examining two replicate sections taken from the mid-point of the

petiole. Total number of hyphae in five randomly selected areas of 4 x 10^3 μm^2 in both these sections were counted.

Table 13: Colonization and external symptoms in oil palm petioles 8 d post inoculation with *F.o.e.*

CLONE	TREATMENT	SYMPTOMS*	FUNGAL GROWTH* IN PETIOLES
WR	Infected	0.5 a	20.3 b
	Control	0.7 a	0.0 a
WS	Infected	1.8 b	38.2 c
	Control	0.9 a	0.0 a

Values are means of five replicate palms.

Within each column, means followed by the same letter are nsd (P>0.05) using Kruskal Wallis and repeated Mann Whitney tests.

External symptoms (browning) were assessed (see 2.8.3).

*Fungal growth in petioles was assessed by microscopic examination (see text)

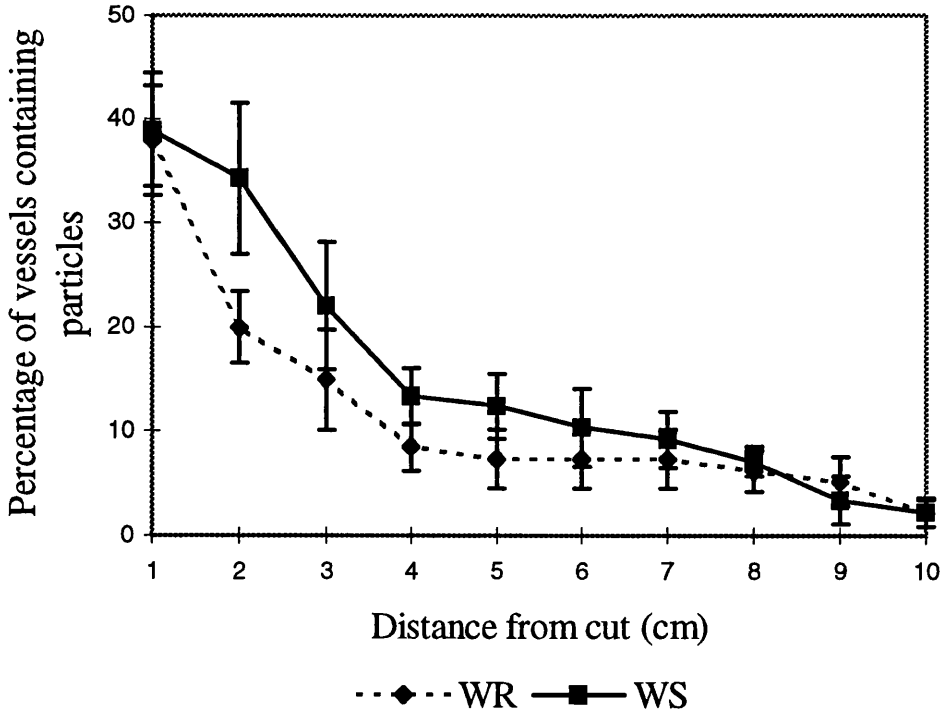
External browning of petioles was first observed in the susceptible clone at the site of inoculation, 4d after inoculation (data not shown). The browning of the petioles intensified and spread rapidly away from the cut end to a significant level and by 8d post inoculation, the majority of petioles were brown and abundant hyphae were observed in cross sections from this clone (Table 13). However, control petioles and the majority of infected petioles from resistant clone remained green and only showed slight external browning at the cut ends and there was less fungal growth in the vessels (Table13).

4.2.2.2 Preformed physical barriers in petioles; determination of vessel lengths.

Previous results in infected roots and petioles from resistant clones showed restricted fungal growth in xylem vessels compared to susceptible clones. Vascular structure such as short vessel lengths could contribute to this expression of resistance to fungal invasion. To clarify this point, vessel lengths in petioles from both clones were determined by the methods previously described (2.10).

Generally,there was no significant difference in vessel lengths between the resistant and susceptible clone; only at 2 cm from the cut end where *ca.* 20% and *ca.* 34% of vessels contained fluorescent particles in resistant and susceptible clones respectively was this difference significant (Fig. 4).

Figure 4: Vessel lengths in petioles of (WR) and (WS) clones revealed by fluorescent particles.



4.2.3 Preformed and induced antifungal compounds from oil palm petioles

4.2.3.1 Antifungal compounds in petiole xylem fluids.

During host invasion by vascular pathogens, the advancing parasites in the xylem stream may meet preformed or induced barriers, both physical and chemical which can potentially limit the extent of colonization. The antifungal properties of xylem fluids from oil palm petioles, uninfected and infected with *F.o.e.* were studied from a resistant clone.

Petioles from five plants (one petiole per plant) were inoculated as previously (2.6) and incubated for 4 d before extraction of xylem fluid. Petioles inoculated with sterile distilled water served as controls. In a preliminary extraction, a pressure bomb was used to force fluids from the xylem but it was found that extraction by syringe allowed faster sampling and without having to dismantle the bomb apparatus. Ethyl acetate was used initially to flush through each petiole (2.12.1). After 12h incubation in a humid chamber at 25°C, spore germination and germ tube growth were assessed (2.13.1).

Table 14: Effect of petiole xylem fluid on growth of F.o.e. conidia

TREATMENT	SPORE GERMINATION (%)	GERM TUBE LENGTH (µM)
Uninoculated	98 a	34.1 a
Inoculated	95 a	17.5 b
Solvent	95 a	35.9 a
Water	9 a	37.3 a

Values represent the mean of five replicate plants, 1 petiole per palm and observation of 100 spores per treatment. Values with the same letter are nsd ($P>0.05$) using Kruskal Wallis and Mann Whitney repeated U tests.

Germ tube length ranged from 17.5 - 37.3 μm and was significantly reduced in fluid from infected petioles while germ tube growth in extracts from uninoculated petioles, solvent and water controls were not significantly different; none of the treatments caused significant inhibition of spore-germination (Table 14).

Control petioles and the majority of infected petioles from the resistant clone remained green and only showed slight external browning at the cut ends and there was restricted fungal growth in the vessels.

4.2.3.2 Optimization for extraction of antifungal compound(s) in petiole xylem fluids of resistant palms.

It was evident that xylem fluids in infected petioles were antifungal. In order to optimize extraction and as a preliminary step to characterization of antifungal compound(s), solvents ranging from polar to non-polar were used for extraction. Petioles from the resistant clone were inoculated and incubated for 4d as described above. Extraction of xylem fluid was conducted by flushing the xylem in succession with solvents of increasing polarity. The solvents were used in the following order: Petroleum ether (PE), Diethylether (DE), Ethyl acetate (EA), Methanol (100%). The resulting extracts were dried and assayed for antifungal activity against *F.o.e.* conidia as previously described.

Table 15: Antifungal activity of petiole xylem fluids extracted with four solvents.

SOLVENT	TREATMENT	% SPORE GERMINATION	GERM TUBE LENGTH (μM)
Petroleum ether	Uninoculated	97.7 a	61.5 bc
	Inoculated	98.0 a	73.8 b
	Solvent	98.0 a	37.5 d
Diethyl ether	Uninoculated	98.3 a	61.3 bc
	Inoculated	97.7 a	44.0 d
	Solvent	99.0 a	36.9d
Ethyl Acetate	Uninoculated	98.0 a	64.8 bc
	Inoculated	97.0 a	73.8 b
	Solvent	99.0 a	79.5 ab
Methanol	Uninoculated	99.0 a	91.5 a
	Inoculated	98.7 a	109.3 a
	Solvent	99.0 a	66.0 b
Water		99.0 a	62.4 bc

Values represent the mean of five replicate plants, 1 petiole per plant, and 100 observations per treatment. Within each column values with the same letter are nsd ($P>0.05$) using Kruskal Wallis and repeated Mann Whitney tests.

Germ tube length generally ranged from 36.9 - 109.3 μm; a reduction was apparent in extracts from inoculated plants with D.E. (mean 44.0 μm) which was significantly less than the growth from uninoculated xylem extracts (mean 61.3 μm). However, least growth

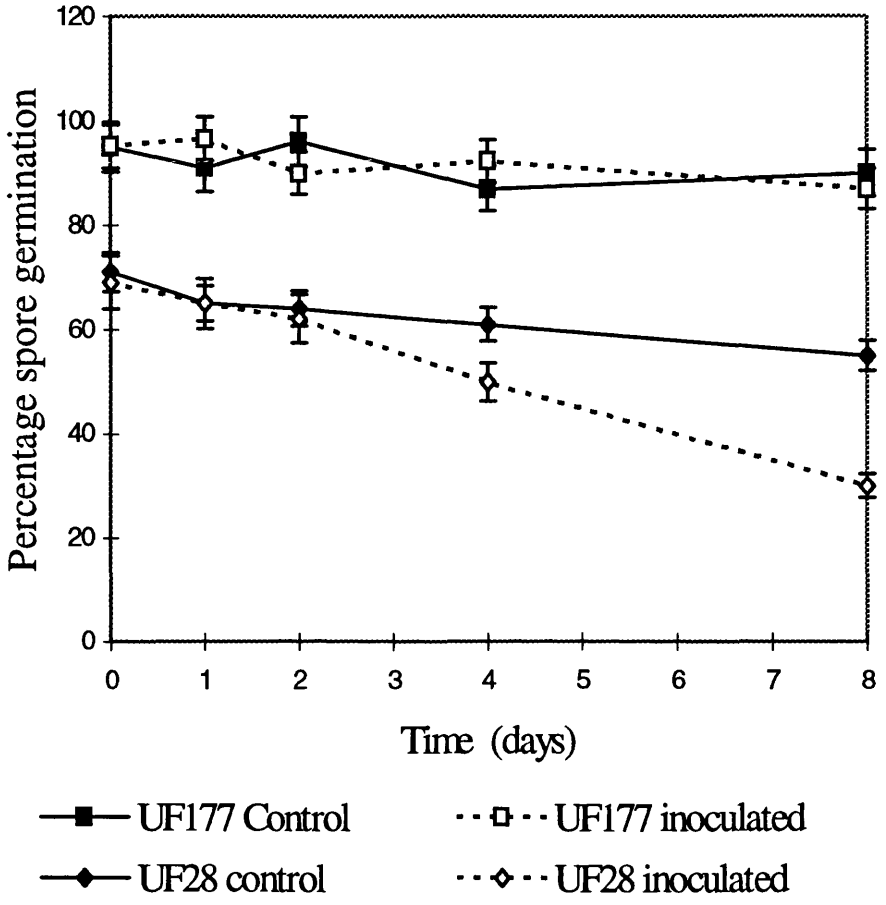
occurred in the D.E. solvent control (mean 36.9 μm); although this was not significantly different from the extracts from inoculated plants, this value was below those of other solvent controls (Table 15). This was a feature common to PE and DE and suggested antimicrobial impurities therein. Growth was slightly enhanced, (73.8 - 109.3 μm) with methanol, EA and PE extracts from inoculated plants compared to those from uninoculated petioles (61.5 - 91.5 μm) (Table 15). This suggested that growth promoting factor(s) in the host were rendered more available following infection. None of the treatments caused significant inhibition of spore germination which was 97 - 99% for all treatments (Table 15). Based on these data a higher grade of diethylether(99.7%) was chosen for subsequent extractions.

4.2.3.3 Temporal accumulation of antifungal compound(s) in petiole xylem fluid.

Petioles from five replicate plants of a resistant and a susceptible clone (UF28 and UF177 respectively) were inoculated as described before (2.12.2).. Antifungal activity on conidia was assessed 0,1,2,4 and 8 d days after inoculation.

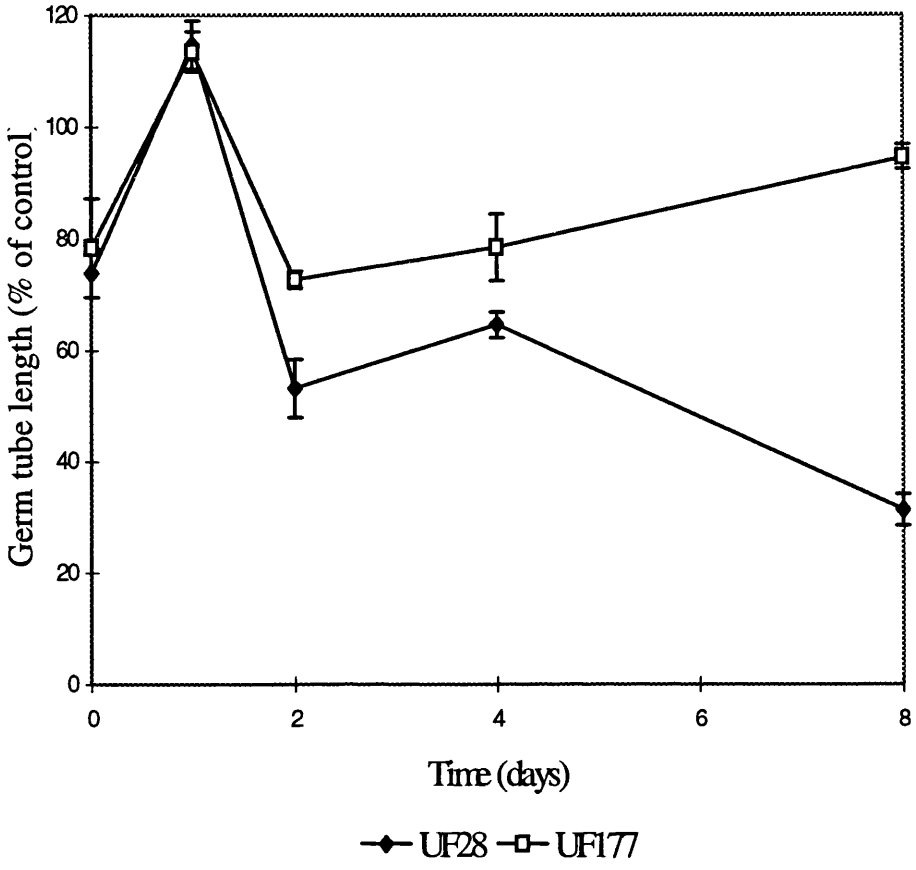
Inhibition of spore germination in extracts (control and inoculated) from xylem fluids of the resistant clone was evident even before inoculation (time zero) compared to the susceptible (Fig. 5a). This indicated the existence of preformed antifungal compounds in the resistant line. Antifungal activity (vs germination) increased with time in the resistant clone, thus 8 d after inoculation, spore germination was *ca.* 30% in extracts from inoculated petioles of the resistant clone and *ca.* 55% from uninoculated controls (Fig.5a). In contrast, little antifungal activity was apparent from either inoculated or control petioles of the susceptible clone; 8d extracts allowed 85 - 90% germination. Inhibition of spore germination was significantly greater in extracts from both inoculated and uninoculated petioles of resistant compared to the susceptible clone at all times.

Figure. 5a:Temporal accumulation of antifungal compounds(s) in petiole xylem fluid; effect on spore germination.



Values are the mean (± SE) of five replicates.

Figure5b:Temporal accumulation of antifungal compounds(s) in petiole xylem fluid;effect on germ tube elongation.



Values are the mean (± SE) of five replicates.

Also, extracts from inoculated petioles of resistant palms caused significant reduction of germ tube growth (Fig. 5b). Following inoculation, growth was reduced in extracts from the resistant line by 47% (compared to the uninoculated control) but only by 7% in extracts from the susceptible plants (Fig. 5b).. Extracts from the resistant clone became increasingly inhibitory so that by 8 d germ tube growth was reduced by 68% compared to controls in contrast to only 6 % reduction in the susceptible clone.

4.2.4 Optimization for extraction of antifungal compounds from petiole tissue of resistant palms.

It was evident from previous experiments that antifungal compounds accumulate in xylem of the resistant clone. However, activities were low and probably insufficient to facilitate separation and characterization. Also, in view of the synthesis of phytoalexins by living plant cells (Bailey, 1982), it was decided to amend the inoculation procedure to infiltrate intercellular spaces as well as xylem of petioles in order to challenge many more cells with *F.o.e.* Extraction from the entire petiole was then performed, more in line with other model systems developed for studies of phytoalexins.

To optimize extraction of antifungal compounds from petiole tissue, segments (2.5 cm) of petioles from five resistant palms (one petiole per plant) were vacuum infiltrated with 0.5 ml of spore suspension (3×10^7 ml^{-1}). Controls received SDW. All treatments were incubated in humid chambers at 28°C for 8d. Extraction for antifungal compounds from petioles was conducted as described before (2.12.2.) Spore germination and germ tube growth were assessed as above.

Table 16:Antifungal activity of petiole tissue extracted with four solvents.

EXTRACTION SOLVENT	TREATMENT	GERMINATION %	GERMTUBE LENGTH (µM)
Petroleum ether	Uninoculated	98.7 a	23.6 a
	Inoculated	99.0 a	23.4 a
	Solvent	86.6 a	12.7 b
Diethyl ether	Uninoculated	56.7 b	11.4 b
	Inoculated	0 c	0 c
	Solvent	98.0 a	22.2 a
Ethyl acetate	Uninoculate	85.0 a	15.6 b
	Inoculated	32.7 b	10.8 b
	Solvent	90.0 a	25.6 a
Methanol	Uninoculated	99.0 a	36.8 a
	Inoculated	33.0 b	13.2 b
	Solvent	99.0 a	35.6 a
Water		90.5a	32.0 a

Values represent the mean of five replicate plants, one petiole per plant and 100 observations per treatment. Within each column values with the same letter are nsd ($P>0.05$) using Kruskal Wallis and Mann-Whitney repeated tests.

Tissue extracts with D.E. from inoculated petioles caused complete inhibition of spore germination. This compares with *ca* 57% germination in extracts from uninoculated

petioles or 98% in the solvent control. In addition to this high level of induced antifungal activity, inhibition by the uninoculated control may indicate a wound response or preformed activity (Table 16).

Similarly, some inhibition was apparent with E.A. and methanol extracts from inoculated petioles compared to extracts from uninoculated petioles or solvent controls (Table 16). Extracts with P.E. enhanced spore germination as compared to water controls. Reduction in germ tube growth in D.E. extracts mirrored the results for spore germination. Other solvents were similarly less effective than D.E. There was significant reduction in growth in P.E. solvent controls (*ca.* 13µm)compared to 32µm in water. Consequently, diethyl ether was chosen for subsequent extractions because: i) it resulted in greatest antifungal activity, ii) its high volatility facilitated evaporation of extracts to dryness.

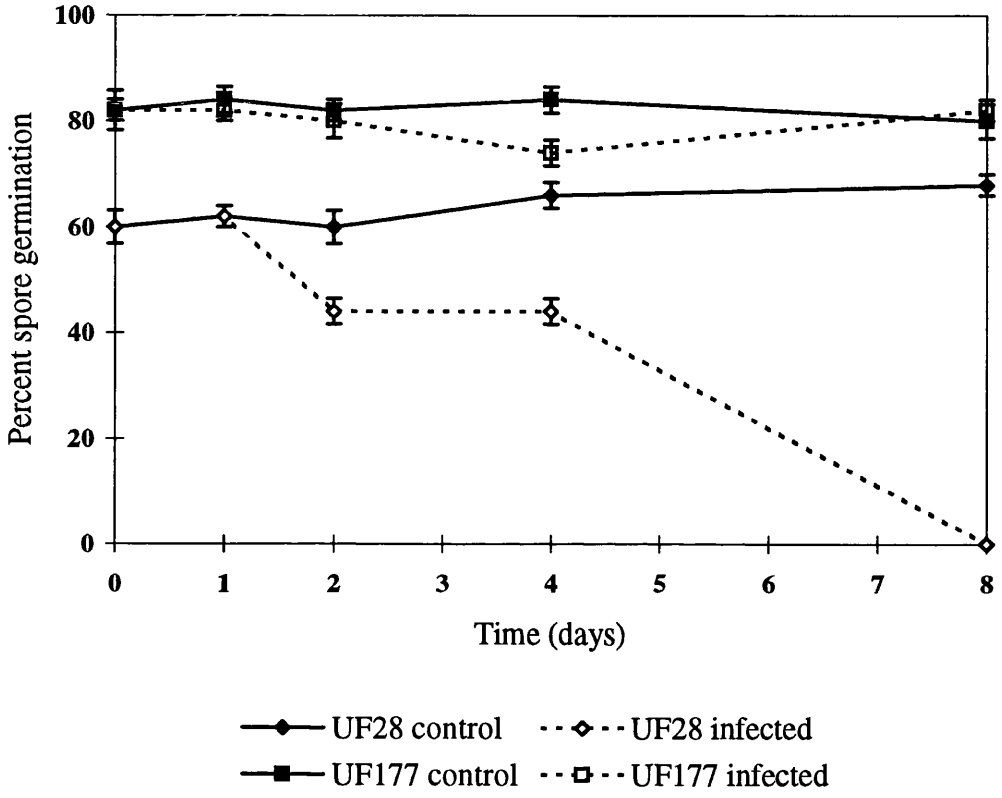
4.2.4.1 Temporal accumulation of antifungal compounds in resistant and susceptible petiole tissue.

It was evident from previous experiments that 8d post inoculation, antifungal compound(s) in a resistant clone accumulated in xylem fluid and was completely fungistatic in tissue extracts. The comparative rate and level of accumulation of antifungal compound(s) in both resistant and susceptible palms were unknown and were consequently investigated. Extraction and assessment of activity of antifungal compounds on spore germination and germ tube growth were conducted as described in (2.12.3 and 2.13.1).

Antifungal activity increased with time in infected petioles of the resistant clone and by 8d the extracts caused complete inhibition of spore germination (Fig. 6a). As well as induced antifungal activity, significantly lower germination occurrred at time zero in the resistant line compared to the susceptible one, indicative of preformed antifungal factors. No

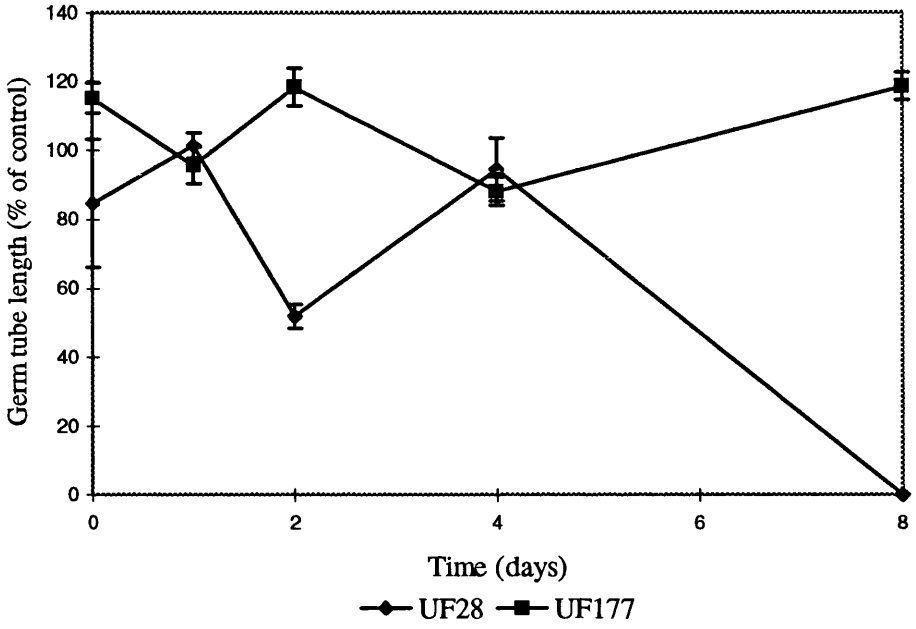
inhibition of spore germination was observed in extracts from either inoculated or uninoculated petioles of the susceptible clone. (Fig 6a)

Figure 6a:Temporal accumulation of antifungal compound(s) in petiole extracts; effect on spore germination.



Values are the mean (± SE) of five replicates.

Figure 6b: Temporal accumulation of antifungal compund(s) in petiole extracts; effect on germ tube elongation.



Values are the mean (± SE) of five replicates.

Germ tube length of infected plants expressed as percentage of uninoculated controls.

Although after 2d, germ tube length was significantly shorter (46% of controls) in extracts from resistant petioles compared to that in extracts from susceptible clone (118% of control), by 4d there was no significant difference between them. However, by 8d, the extract was completely inhibitory. In contrast, germ tube growth was generally enhanced (cf. uninoculated controls) in extracts from the susceptible clone (Fig. 6b).

4.2.5 Antifungal compounds from oil palm roots.

Roots are the likely site of entry for *F.o.e.* from soil and it may be in roots where resistance is first expressed. It is evident from this work (Scetion 3) that eight months after inoculation, the amount of *F.o.e.* reisolated from pseudobulb of the resistant clone

UF28 (WR clone 2) was less frequent and in some plants absent compared with the significantly higher amounts recovered from UF177 (WS clone). Therefore, experiments comparable to those with petioles were performed in order to assess the potential of root tissue for production of antifungal compound(s).

Selected root segments from rsistant and susceptible clones were infiltrated with 0.5 ml of spore suspension (3 x 10^7 spores ml^{-1}) and extraction for antifungal compounds was conducted as before.

Table 17: Antifungal activity of ether extracts from resistant and susceptible oil palm roots.

WILT STATUS	TREATMENT	% SPORE GERMINATION
WR clone	Uninoculated	0
(UF 28)	Inoculated	0
WS clone	Uninoculated	60 b
UF 177)	Inoculated	70 b
	Solvent Control	91.7 a
	Water Control	93.4 a

Values represent the mean of five replicate plants (1 root/plant).Within each column, values with the same letter are nsd using Kruskall Wallis and repeated Mann-Whitney tests.

Ether extracts from tissues of inoculated and uninoculated roots of the resistant clone caused complete inhinition of spore germination (Table 17). Also, both inoculated and uninoculated susceptible clone showed significant inhibition of spore germination compared to water or solvent controls but there was no significant difference between these extracts (inoculated vs uninoculated WS).

4.2.6 Response of petioles from three susceptible and three resistant clones to infiltration with conidia of *F.o.e.*

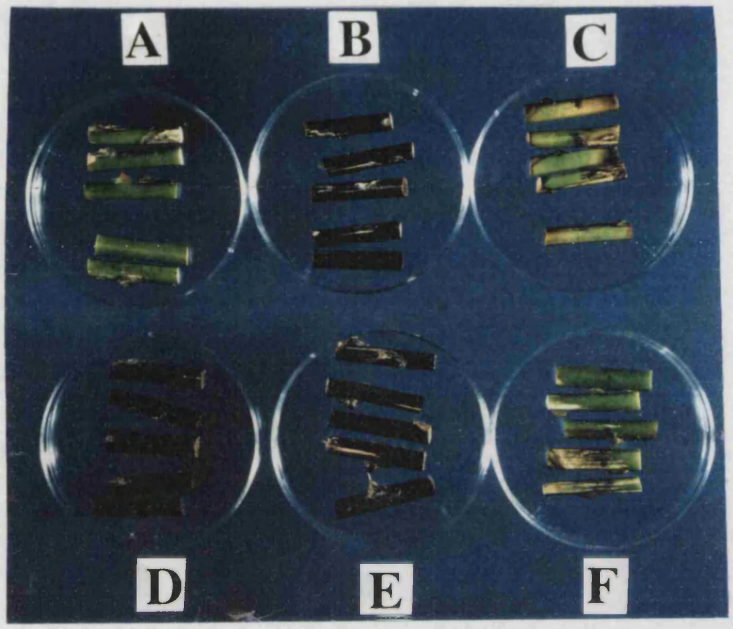
It was evident from previous work that resistance was expressed in infused resistant petioles by the retention of green colour and high level accumulations of antifungal compound(s). However, only one clone representing susceptibility and resistance had so far been studied and this needed to be extended.

The objective of this experiment was therefore to compare petioles infused with *F.o.e.* from six clones, three resistant and three susceptible based on assessments of external symptoms, fungal colonization and accumulation of antifungal compounds. All assessments were performed 'blind' to remove possible subjectivity.

Inoculation, and assessment of symptoms and fungal colonization were conducted as given in Materials and Methods (2.7, 2.13.1 and 4.2.2.1).

In susceptible lines, external browning of the petioles began after 4d, first at the cut end at the point of infiltration. Browning then progressed along these petioles and was severe by 8d. At this time, varietal differences based on external symptom were obvious as resistant clones remained green and intact whilst the petioles from the susceptible clones turned brown and became soft (Plate 3). Most spores did not germinate in petiole tissue from R_1 and R_2 (Table 18).

Plate 3: External symptoms of petioles from three susceptible and three resistant clones following inoculation.



External symptoms on petioles of 6 clones following infusion with conidia of *F.o.e.* A,C and F are WR; B, D and E are WS clones.

Table 18: Fungal colonization and antifungal activity in infused petioles of six clones

CLONE	WILT STATUS	FUNGAL GROWTH IN PETIOLE (µM)	SPORE GERMINATION (%)+	GERM-TUBE GROWTH (µM) *
R_1	Highly resistant	2.9 a	0	0
R_2	Resistant	3.7 a	15 a	4.7 a
R_3	Moderately resistant	19.6 ab	25 ab	10.7 ab
S_1	Moderately susceptible	29.7 ab	67 bc	20.2 b
S_2	Susceptible	36.5 bc	73 c	20.3 b
S_3	Highlysusceptible	54.8 c	85 c	23.1 b

Values represent the mean of six replicate plants, one petiole per palm and observation of five sections per petiole.

+percentage germination as compared to 86% for water control and 88% for solvent control. * germ tube length as compared to 24.5 µm for water control and 22.8 µm for solvent control.

Within each column values with the same letter are nsd (P>0.05) using Kruskall Wallis and repeated Mann Whitney tests

Diethyl ether extracts from petiole tissue of the most resistant clone (R_1) caused complete inhibition of spore germination but antifungal activity in resistant clone R_3 was lower and similar to that from some susceptible clones based on fungal growth in petioles, spore germination and germ tube growth (Table 18). However, in general, spore germination was significantly reduced in extracts from resistant plants compared to growth in extracts from susceptible plants which was nsd from controls.

Therefore, the general responses of the six clones based on external symptoms, fungal growth in petioles and effects of petiole extracts on spore germination and germ tube growth were correlated to varietal differences in terms of disease resistance as determined by conventional soil inoculation (Section 3).

4.2.7 Sensitivity of four *F.o.e.* isolates to antifungal compound(s) from palm petioles.

To date, antifungal effects of extracts from petiole tissue of resistant and susceptible palms had been studied on one isolate only, F_3. However, the relative toxicity of these extracts (as different concentrations) and the sensitivity of other oil palm isolates of *F.o.e.* of varying levels of aggressiveness were unknown.

To investigate these interactions, petioles from the highly resistant clone UF196 (clone R_1) were inoculated and antifungal compounds extracted as described before. To determine their fungitoxicity, aliquots of th etcts ranging from 15 - 60 µl were placed in deep well slides. This range was known to be efetive from the previous experiments. The extracts

were evaporated to dryness and inoculated with conidia. Spore germination and germ tube growth of 3 pathogenic (16F, F_3 and OPC_1) *F.o.e.* isolates and one non-pathogenic (C_2) *F. oxysporum* isolate were assessed with tissue extracts made 4 and 8 d post infiltration.

Table 19 a:Effect of tissue extract concentrations from 4 d infected petioles on spore germination (%) of 4 *F.o.e.* isolates.

	ISOLATES			
EXTRACT CONC (µl)	**16 F**	**F_3**	**OPC_1**	**C_2**
0	92 a(i)	98 a(i)	97 a (i)	98 a(i)
15	82 a(i)	96 a(i)	97 a(i)	55 b(ii)
30	87 a(i)	84 a(i)	77.5 ab(i)	45 b(ii)
45	42.8 b(i)	72.5 ab(i)	60 ab(i)	15 c(ii)
60	47.5 b(i)	40 c(i)	55.5 b(i)	15 c(ii)

Table 19b:Effect of tissue extract concentrations from 8 d infected petioles on spore germination (%) of 4 *F.o.e.* isolates.

	ISOLATES			
EXTRACT CONC. (µl)	**16 F**	**F_3**	**OPC_1**	**C_2**
0	92 a(i)	97 a(i)	97 a(i)	97 (i)
15	0.5 b(iii)	65 (ii)	76.5 a(i)	0
30	0	10 c	0	0
45	0	0	0	0
60	0	0	0	0

Values represent mean of five replicte plants.

Within each column values with the same letter are nsd (P>0.05).

Within each row, values with the same number are not sd (P>0.05) using Kruskal Wallis and Mann-Whitney repeat tests.

Germination of the non pathogenic isolate ,C_2 was inhibited at only 15ul of the 4d extract while the aggressive isolates were only inhibited by *ca* 45 ul (Table 19a).here was no significant difference between the aggressive isolates ; only C_2 was significantly more inhibited at any one concentration (Table 19 a). Germination of all isolates was completely inhibited at 45-60ul of 8d extract and only OPC1 erminated at 30ul. (Table 19 b). At 15ul, C_2 was completely inhibited but isolate sensitivity was not directly related to aggressiveness as the ranking order was 16F, F_3> OPC_1.

Germ tube growth in 4d extracts was reduced for C_2 by 15 µl, 30ul inhibted F_3 and all isolates germinated less in 45ul except OPC_1 which was apparently unaffected at any concentration (Table 19c). Eight day post inoculation extracts mirrored the sensitivity of germination of each isolate with significant reduction in germ tube length for all at 15 µl except F_3 which was inhibited at 30 µl (Table 19d).

Overall, the non-pathogenic isolate, C_2, was more sensitive than the pathogenic isolates but there was no consistent link between phytoalexin tolerance and aggressiveness.

Table 19c:Effect of tissue extract concentrations from 4d infected petioles on germ tube growth (µm) of four *F.o.e.* isolates

	ISOLATES			
Extract conc. (µl)	**16 F**	**F_3**	**OPC_1**	**C_2**
0	21.0 a(i)	31.6 a(i)	34.5 a(i)	34.5 a(i)
15	17.2 a(i)	24.4 a(i)	24.0 a(i)	16.3 b(i)
30	19.3 a(i)	11.6 b(i)	25.6 a(i)	14.9 b(ii)
45	5.8 b(iii)	13.3 b(ii)	26.6 a(i)	4.6 c(iii)
60	10.9 b(ii)	12.6 b(ii)	22.8 a(i)	8.1 bc(ii)

Values represent mean of five replicate plants. Within each column values with the same letter are nsd (P>0.05). Within each row, values with the same number are not sd (P>0.05) using Kruskal Wallis and Mann-Whitney repeat tests.

Table 19d: Effect of tissue extract concentrations from 8 d infected petioles on germ tube growth (µm) of four *F.o.e.* isolates.

	ISOLATE			
Extract conc. (µl)	**16 F**	**F_3**	**OPC_1**	**C_2**
0	21.0 a(i)	22.5 a(i)	32.5 a(i)	0
15	2.6 b(ii)	16.1 a(i)	19.8 b(i)	0
30	0	3.5 b	0	0
45	0	0	0	0
60	0	0	0	0

Values represent mean of five replicate plants.

Within each column values with the same letter are nsd (P>0.05).

Within each row, values with the same number are not sd (P>0.05).

[Using Kruskal Wallis and Mann-Whitney repeat tests].

4.2.8 Attempted separation and identification of antifungal compounds by Thin Layer Chromatography (t.l.c.).

It remained to separate, purify and identify the induced antifungal factor(s) in oil palm petioles or roots; 8 d post inoculation was selected for extraction as this was the time of maximum accumulation of antifungal activity.

Several solvent systems were used in an attempt to separate the compounds on t.l.c. (2.13.2). However, only system C (DE:methanol:PE,6:1:3) run for 15 cm gave reasonable resolution revealing fluorescent bands under UV light (254 nm). The plates were then sprayed with a dense spore suspension of *Cladosporium herbarum, Verticillium dahliae* or *Fusarium oxysporum* f. sp. *eleaidis* and the latter was visualized under light microscope. In all cases, the dark grey/green mycelia of *C. herbarum,* the black mass of *V. dahliae* or white mass of *F. oxysporum* f. sp. *eleaidis* mycelia colonized the entire silica gel plates but revealed no light areas indicative of zones of inhibition. In contrast, the same extracts when slide bioassayed caused complete inhibition of spore germination with all fungal test organisms.

Other plates were sprayed with either vanillin or diazotized p-nitroaniline reagents. Compounds containing phenolic hydroxyl groups which have a free *para* or *ortho* position and which do not contain strongly deactivating substituents or sterically hindering groups, react with diazotized amines (at a suitable pH) to give coloured azodyes (Dawson *et al.*, 1986). Deep orange/brown bands at Rf 0.68 - 0.70 indicated the presence of phenolic

compounds. The extract further contained one fluorescent yellow band with Rf value 0.85 corresponding to that of cinnamic acid (Rf 0.93) (Vernenghi *et al.*,1987). Vanillin did not reveal any pink colour indicative of phenolic compounds. Tissue extracts from either petioles or roots reacted similarly.

4.2.9 Antifungal activity of extracted tannins.

Although this work had clearly demonstrated the presence of antifungal compound(s) in resistant oil palms, identification of these substances remained unresolved. Based on the literature available on tannins showing antifungal activity in some plants, a preliminary test was conducted to investigate if antifungal tannins may derive from the phenolics that had been indicated on t.l.c. from extracts 8 d post inoculation.

Extraction from petiole tissue (inoculation 8d) was conducted (2.12.3) and tested for fungitoxicity. Fifty microlitres of the extracted samples were spotted on t.l.c. plates and run on t.l.c. plates in a mixture of diethyl ether : methanol (6:1).

There were no inhibitory zones where *Cladosporum, Verticillium* or *Fusarium* had been sprayed. In contrast, these extracts when slide bioassayed caused complete inhibition of spore germination with all three tested organisms.

In summary, it was shown that extracts from both petiole xylem fluid and tissue of a resistant palm clone contained preformed antifungal compound(s) and subsequent to inoculation, induced antifungal activity was also observed. Accumulation of antifungal compound(s) was maximal between 4 - 8 d after infiltration with *F.o.e.* conidia. Extracts from roots of resistant plants also contained induced antifungal compound(s),

Phenolic compounds were detected using t.l.c and one of the compounds had the same R_f as cinnamic acid. However, no inhibition zones occurred when plates were sprayed with

Cladosporium, Verticillium or which may indicate inactivation or binding of antifungal activity on the silica. Extracted tannins also were not detectable by t.l.c. bioassay in spite of inhibiting germination with slide bioassay.

There was a clear correlation between disease resistance of six clones and external symptoms, fungal colonization and accumulation of antifungal compound(s) in petioles infiltrated with *F.o.e.* conidia; this system may offer a rapid screen for disease resistance.

There was no clear relationship between aggressivenesss of three *F.o.e.* isolates and tolerance to antifungal extracts, but a non-pathogenic isolate was more sensitive.

4.2.10. Resistance related enzymes in infected oil palm petioles.

4.2.10.1 Detection of Phenylalanine ammonia-lyase (PAL).

In the attempt to identify inhibitory factor(s) in extracts from oil palm petiole or root tissue, there was an indication given by brown discolouration of phenolic compounds accumulating in infected palm petioles. Phenylalanine ammonia-lyase (PAL) is a key biosynthetic enzyme at the head of the phenolic biosynthetic pathways. It converts aromatic amino acids into phenolic precursors some of which are involved in the biosynthesis of lignin and production of certain phytoalexins. This enzyme can be synthesized very rapidly *de novo* in resistance responses thus its induction could reflect activation of numerous defence responses. A study of the degree of its induction might therefore provide a relevant indicator of defence gene activation.

Frozen petioles from inoculated and uninoculated segments were comminuted in liquid nitrogen. The homogenate was extracted and PAL assayed as described in 2.14 and 2.15.

The activity of PAL was measured in extracts from resistant and susceptible petioles sampled at intervals of 2d and 4d following wounding or wounding and immediate inoculation with a suspension of *F.o.e.*

Table 20a:Phenylalanine ammonia-lyase activity (in nkats) from inoculated petioles.

	INOCULATED		**UNINOCULATED**	
CLONE	2 d	4 d	2 d	4 d
Resistant	10.0 a(i)	9.0 a(i)	10.0 a(i)	7.0 a(i)
Susceptible	4.0 b(i)	4.0 b(i)	3.4 b(i)	3.2 b(i)

Values represent mean of five replicate plants.

Within each column values with the same letter are nsd (P>0.05).

Within each row values with the same number are nsd using Kruskal Wallis and repeated Mann Whitney tests.

The level of activity in the resistant clone (±*F.o.e.*) remained significantly higher than in the susceptible clone throughout the experiment. However, no increase in activity was observed in resistant or susceptible clones 4d after inoculation or wounding (Table 20 a).

4.2.10.2 Detection of peroxidase and poly-phenoloxidase.

In a further investigation for putative resistance determinants, enzyme assays were conducted for the presence of peroxidases and polyphenoloxidases. Peroxidase is a key enzyme in lignin biosynthesis and polyphenoloxidase has a key role in the oxidation of phenolic compounds to quinones. Both groups of resulting compounds have been linked

frequently with resistance. Extracts from the above inoculated and uninoculated petioles from resistant and susceptible palms were assayed for both enzymes.

Table 20b:Peroxidase activity from the vascular tissue of *F.o.e.* inoculated and uninoculated petioles from resistant and susceptible clones.

		ENZYME ACTIVITY			
CLONE	**TREATMENT**	**DAY 1**	**DAY 2**	**DAY 3**	**DAY 4**
Resistant	Uninoculated	0.026 a (i)	0.033 a(i)	0.022 a(i)	0.022 a (i)
Resistant	Inoculated	0.026 a(i)	0.029 a(i)	0.019 a(i)	0.025 a (i)
Susceptible	Uninoculated	0.028 a(i)	0.037 a(i)	0.031 a(i)	0.032 a (i)
Susceptible	Inoculated	0.026 a(i)	0.036 a(i)	0.034 a(i)	0.038 a (i)

Replication and statistical analysis as for Table 20 a.

Low and similar peroxidase activities were detected in extracts from both inoculated and uninoculated susceptible clones sampled at 2 d and 4 d after inoculation (Table 20 b). These activities were not significantly different from those in the resistant clone and the overall activities in resistant and susceptible clones were very similar throughout the experiment (Table 20 b).

Polyphenoloxidase activity was not detected in any of the samples.

4.2.10.3. Detection of glucanase and chitinase.

Increased activity of ß-(1, 3)-glucanase and chitinase are often found in wounded and infected plant tissues. It is thought that in the absence of endogenous substrates these may act to degrade fungal cell walls and in the process may release elicitor molecules in

infected areas (Keen and Yoshikawa, 1983; Wargo, 1975; Young and Pegg, 1982). The released elicitors from fungal walls may play an important physiological role in the induction of plant defence reactions in response to fungal infection. The objective of this assay was to investigate if these enzymes could be detected from tissue extracts of inoculated and uninoculated palm petioles of resistant and susceptible clones sampled at different time intervals.

Table 21: β-1,3-glucanase and chitinase activity (nkats) from *F.o.e.* inoculated and uninoculated petioles from resistant and susceptible clones.

		ENZYME ACTIVITY							
		GLUCANASE				CHITINASE			
Cultivar	**Treatment**	Day 1	Day 2	Day 3	Day4	Day 1	Day 2	Day 3	Day4
Resistant	Uninoculated	6.5	11.8	17.3	17.3	3.3	14.5	8.6	8.4
	Inoculated	8.5	8.3	17.3	17.3	3.4	14.5	11.8	12.0
Susceptible	Uninoculated	17.5	18.4	18.8	19.5	8.4	8.3	15.3	17.5
	Inoculated	17.3	14.5	17.2	18.5	6.5	8.3	12.0	18.7

Values represent mean of five replicate plants.

Both glucanase and chitinase were detected in resistant and susceptible clones at similar levels of activity (Table 21) but throughout the experiment there was no consistent increase in either activity following inoculation.

4.2.11.: Production of pectin enzymes by *F.o.e. in vitro* and *in vivo.*

4.2.11.1 Time course of enzyme production by isolates 16F and OPC_4 on host cell walls and 1% sodium polypectate.

Previous results from oil palm progenies that had been screened for resistance, in the glasshouse (Section3), with isolates from different oil palm growing areas showed, that the isolates could be ranked with regard to aggressiveness. This was based on both internal symptoms (vascular browning in pseudobulb) and external symptoms (wilt index and leaf chlorosis).

Thus, isolates 16F and 1379 were considered to be highly aggressive, F_3 and Y_1 moderately aggressive, OPC_4 and OPC_1 of low aggressiveness and C_2, non-pathogenic.

Enzymes capable of degrading ce11 walls, notably pectic enzymes, probably play a major role in pathogenesis (Bateman and Basham, 1976; Durrands and Cooper, 1989). Their involvement in pathogenesis may be linked to the vascular disfunction and blockage observed in wilted plants. More significantly in the context of overcoming constitutive and induced resistance, the action of pectic enzymes on the primary wall-middle lamella complex at pit membranes or perforation plates and on the pectic component of vascular gels should facilitate colonization. Endo-pectinases which are (indirectly) toxic to plant cells may be involved in localized death of plant cells (Basham and Bateman, 1975). Thus, variations in production and activities of these enzymes may contribute to the pathogenicity /virulence of the pathogen. These experiments were planned to study resistance in this context.

Preliminary experiments to determine rates of growth and the time course of enzyme production were conducted with isolates 16F and OPC_4 as a prelude to help design an experiment to analyse seven isolates. Basal salts medium containing host cell walls as sole source of carbon in liquid shake cultures were used to induce enzyme synthesis; the insoluble nature of cell walls should not effect catabolite repression (Cooper, 1977). A 5 ml sample was aseptically removed every 24 h up to and including 192 h and stored on ice in the fridge until required for the assay for polygalacturonase (PG), pectin-lyase (PL) and pectin-methylesterase (PME) activity as described in Materials and Methods. A more accurate approach would have been to have determined a growth curve and time course for each isolate because enzyme production may peak at different times dependent on growth rate or even as a component of aggressiveness. Resources and time available, however, did not permit this approach.

Fig 7:Growth of isolate 16F on oil palm cell walls

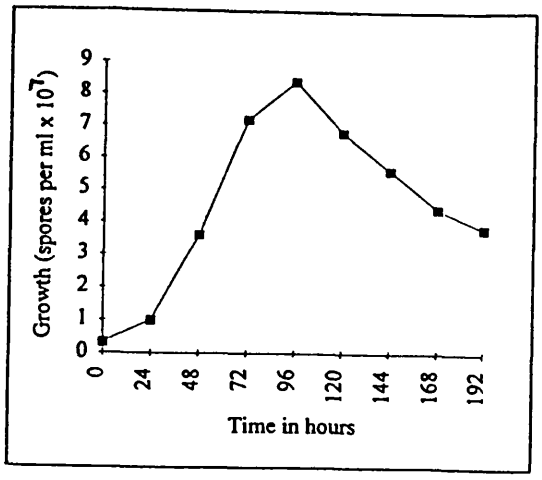


Fig 8: Growth of isolate OPC_4 on oil palm cell walls

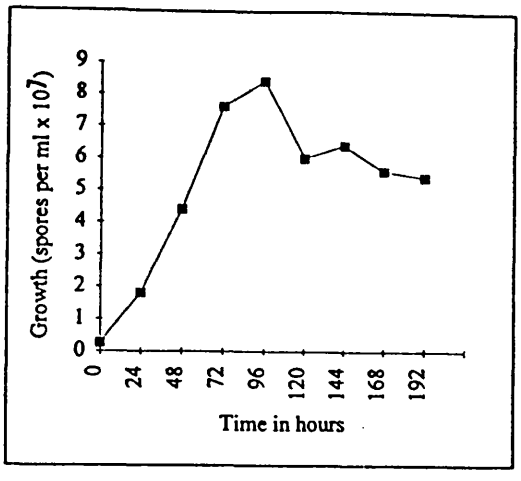


Fig 9 :pH of culture fluid of isolate 16F on oil palm cell walls.

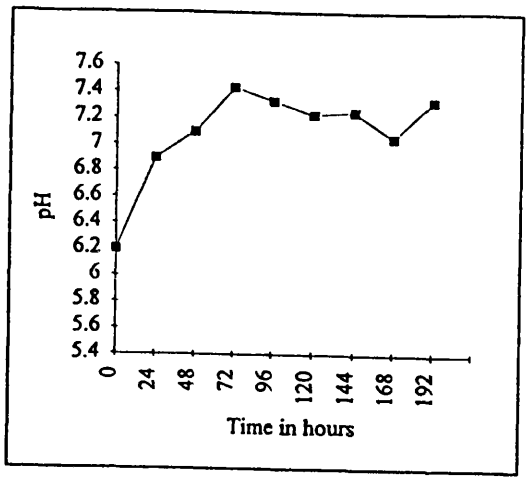


Fig 10: pH of culture fluid of OPC_4 on oil palm cell walls

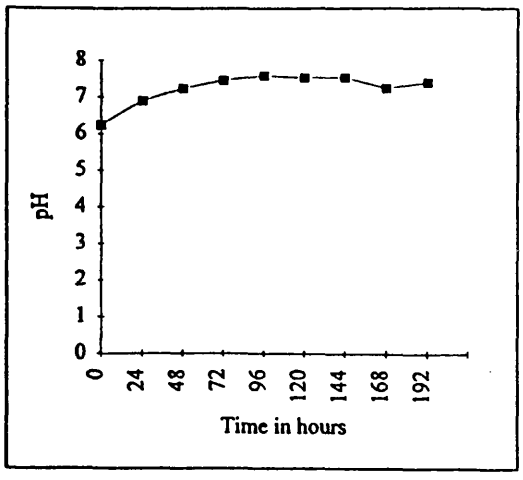


Fig 11: PG production from isolate 16F on oil palm cell walls.

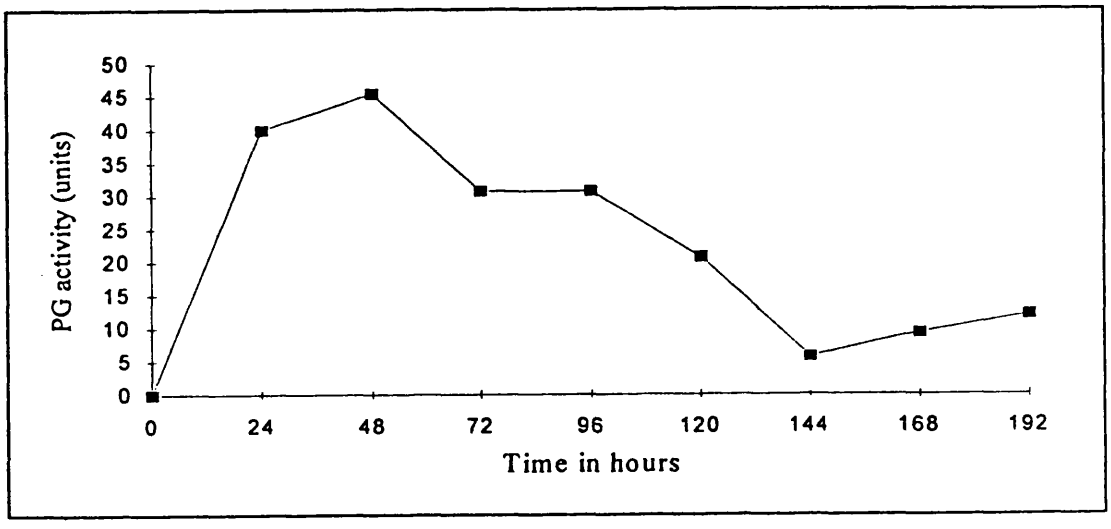


Figure 12: PG production from isolate OPC_4 on oil palm cell walls.

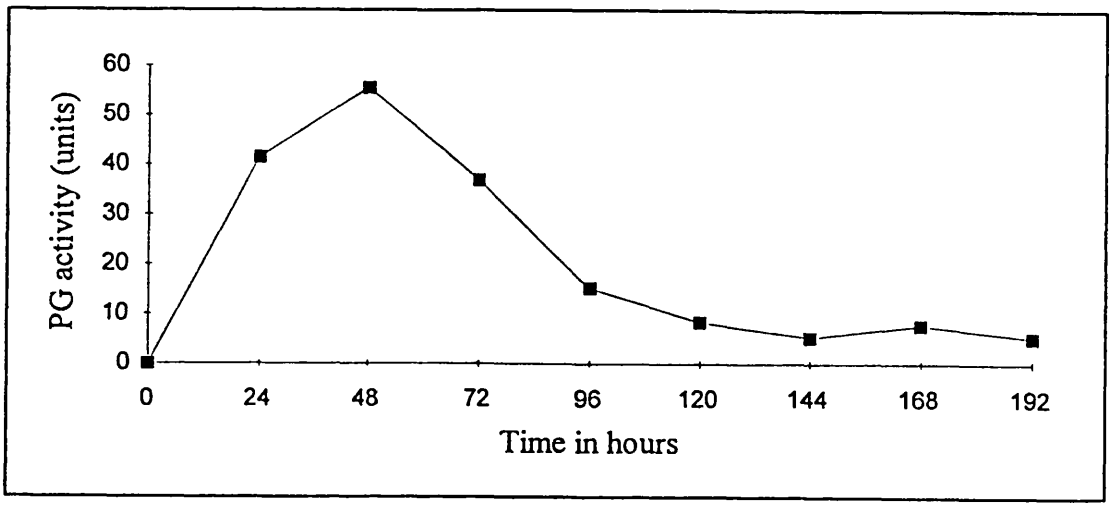


Figure 13: PL production from isolate 16F on oil palm cell walls

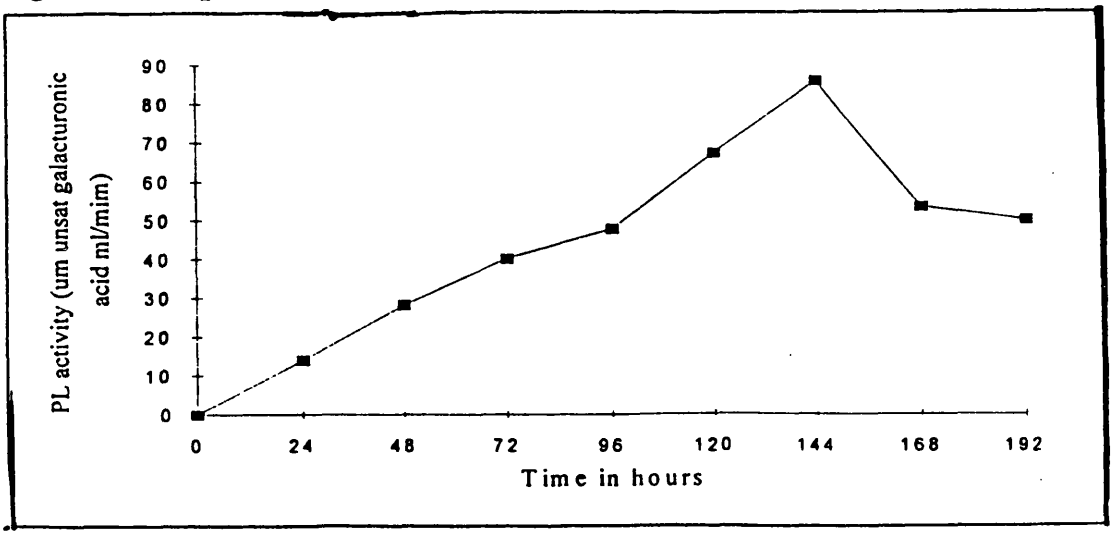
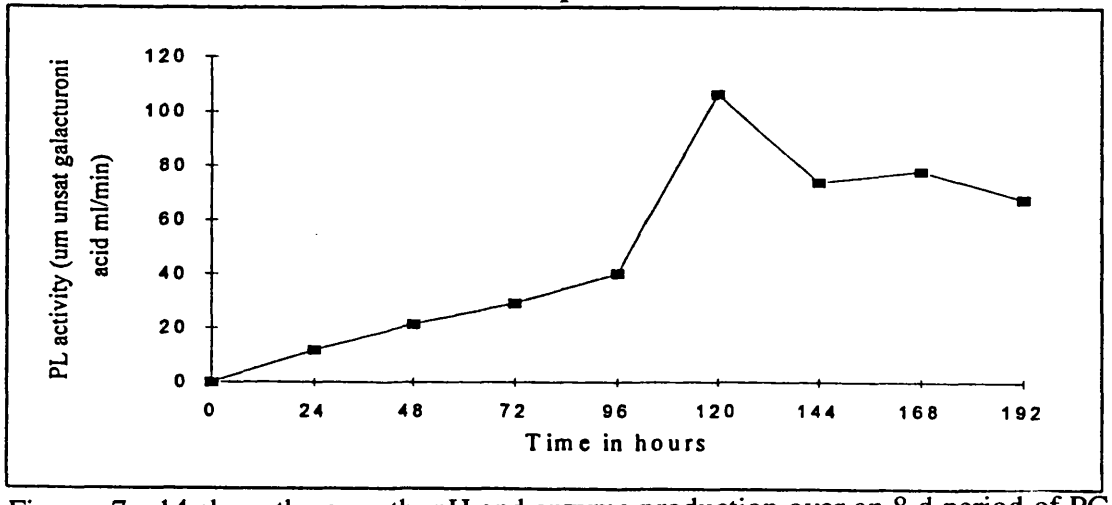


Figure 14: Production from OPC_4 on oil palm cell walls.



Figures 7 - 14 show the growth, pH and enzyme production over an 8 d period of PG and PL by isolates 16F and OPC_4. Growth was rapid and comparable between the two isolates reaching a maximum after 96 h after which cell number declined (Fig. 7 and 8). The pH rose from 6.2 to a maximum of 7.4 and 7.6 for isolates 16F and OPC_4 respectively (Fig. 9 and 10). The similar growth and pH curves suggested that differences in enzyme production would not be markedly influenced by these factors, hence, PG and PL production time for the two isolates should be comparable. Secretion of PG was detected after 24 h and reached a peak after 48 h at *ca.* 45 and 55 units (RVU) for isolates 16F and

OPC_4 respectively (Fig. 11 - 12). Production of PL also occurred by 24 h but reached maximal activity by 140 h for isolate 16F and 120 h for OPC_4 with *ca.* 86 and 106 units respectively. The levels then declined and remained at these levels to the end of sampling (192 h) (Fig. 13 and 14).

Fig. 15: Growth of isolate 16F on 1% pectate.

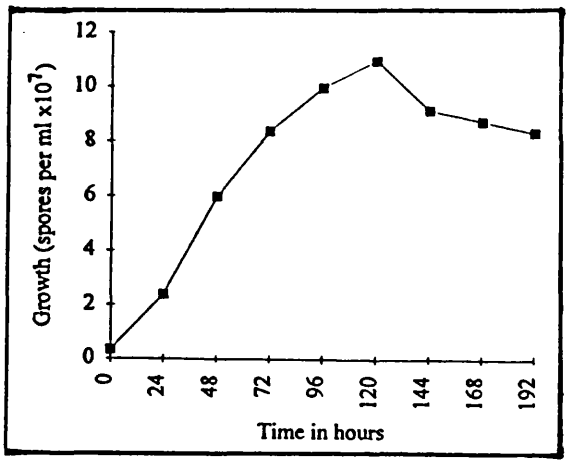


Fig. 16:Growth of isolate OPC_4 on 1% pectate.

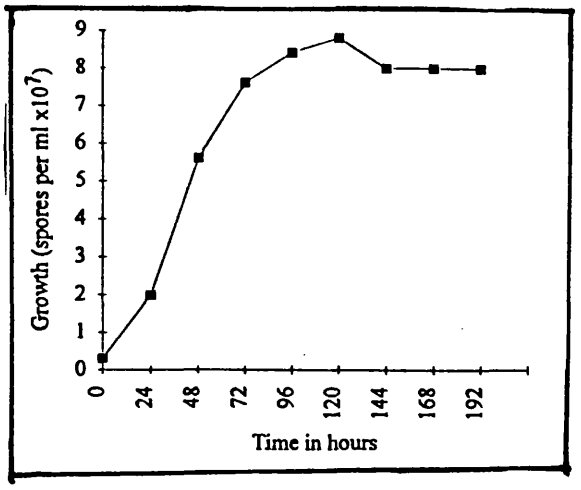


Fig. 17: pH of Culture fluid of isolate 16F on 1% pectate.

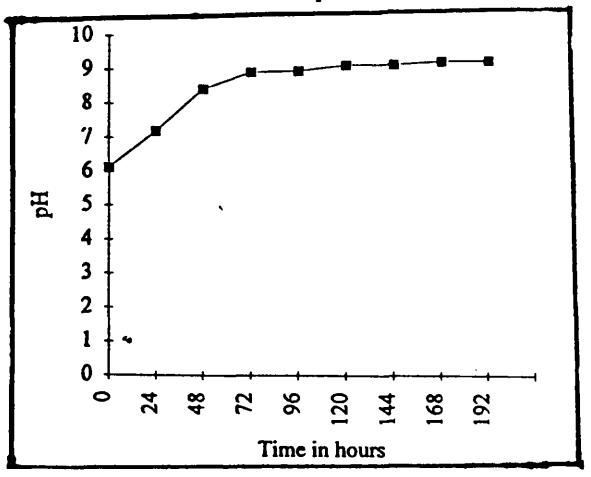
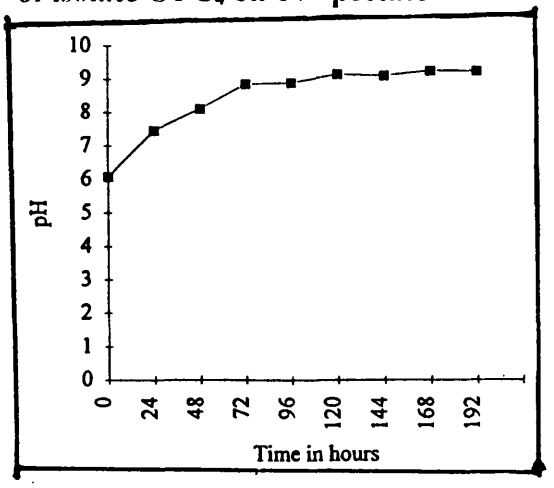


Fig. 18: pH of culture fluid of isolate OPC_4 on 1% pectate.



The two isolates were also grown on 1% pectate for 192 h in an attempt to achieve high levels of PL production. Growth of the isolates was comparable in each case reaching a maximum after 120 h for both isolates (Fig.15 - 20). Growth of isolate 16F was greater

than on cell walls and greater than for OPC_4 (Fig. 15 and 16). The pH of cultures increased from 6.1 to 9.3 and from 6.0 to 9.2 for isolates 16F and OPC_4 respectively (Fig.17 and 18). The higher pH levels obtained when the isolates are grown on pectate compared to cell walls is thought to favour PL accumulation (Cooper, 1977). The similar growth and pH curves thus allowed a comparison of PL production between the isolates.

Production of PL from isolate 16F was detected after 24 h and was maximal (at 282 units) at 72 h then rapidly decreased (Fig. 19). For isolate OPC_4, production of PL was also detected at 24 h but reached maximum of 213 units after 96 h before rapidly decreasing (Fig.20). Levels of PL were *ca.* two times greater than those detected in cultures with cell walls presumably reflecting the influence of substrate (although growth was similar on both) and pH in the culture medium; the latter is more likely to have a major influence (Cooper, 1977). These data indicated likely optimal times for sampling for PG and PL in the larger survey of enzyme production by isolates. Cultures were therefore sampled for PG and PL activity at 72 h and 144 h respectively.

Figure19: PL Production from isolate 16F on 1% pectate.

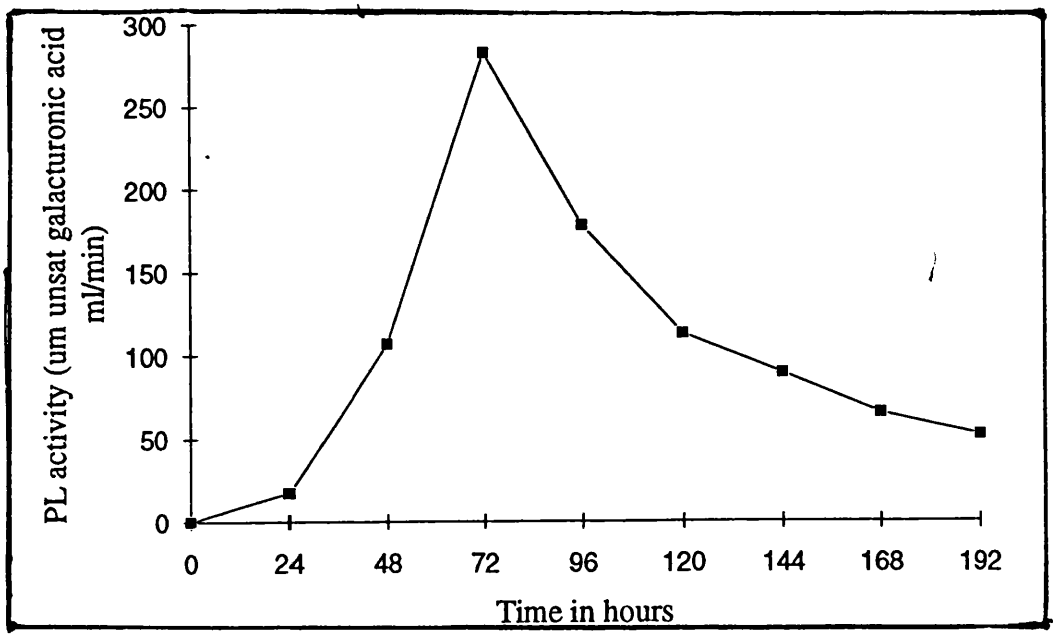
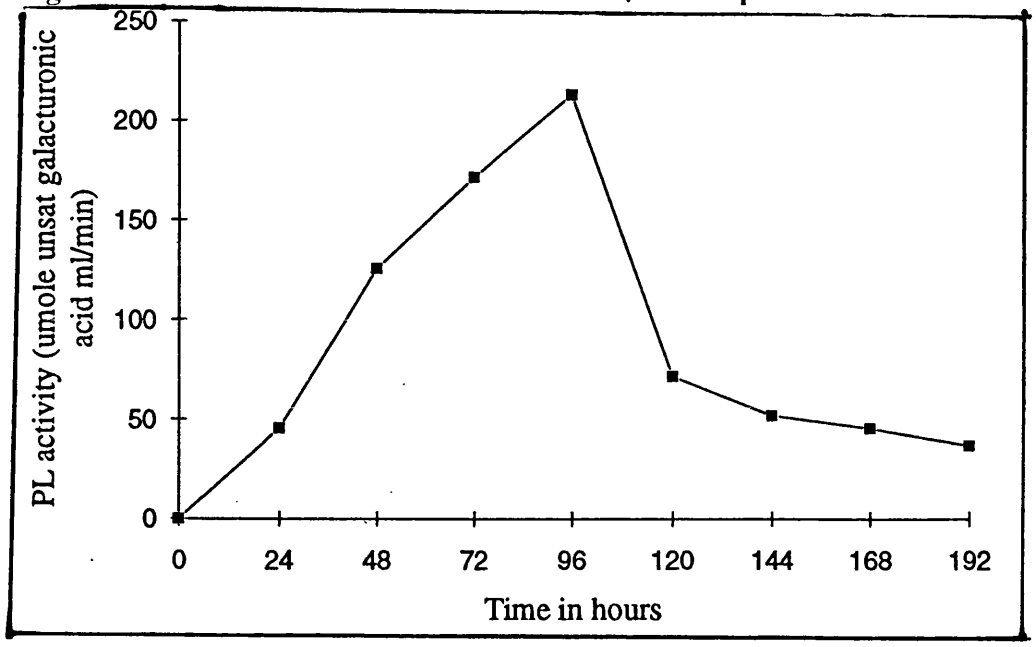


Figure 20: PL Production from isolate OPC_4 on 1% pectate.



4.2.11.2 Pectinmethylesterase (PME) production.

The PME assay repeatedly gave negative results. Several alternatives were tried to the method described which included the use of pH indicators such as phenolphthalein and potassium permanganate instead of pH probes. The culture filtrate and enzyme substrate were also left for varying periods of time up to 6 h.

4.2.11.3 Cup-plate assay for PG.

In order to find an assay more facile than viscometry for determination of PG activity in large numbers of samples, cup-plate assay of culture filtrates was tested. Culture fluid with PG activity (determined by viscometry) resulted in an indistinct opaque halo around the sample site. This differed from the clear halo found around the sample site when commercial endo-PG from *Aspergillus* (Sigma) was used (Fig. 21 A and B). With this enzyme a linear relationship was shown between pectinase units and log zone diameter (Fig. 22). The opaque halos could have been due to cross linking of the polygalaturonic acid due to salts such as Ca^{2+} ions present in the culture filtrate. However, a control comprising salts solutions only did not give an opaque halo. The method was abandoned in

favour of viscometric assay which has an advantage in that it only detects endo-PG activity which is more likely to be a pathogenicity determinant than exo-PG which has minimal effect on plant tissue (Cooper, 1984)

Figure 21: 'A' opaque halo obtained using culture filtrate samples and 'B' clear halos with commercial PG.

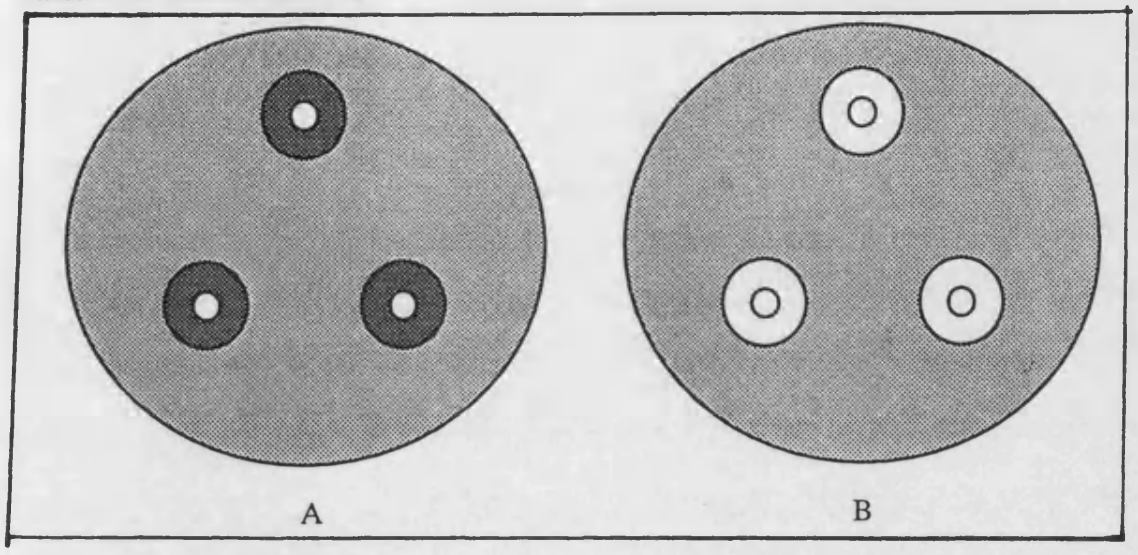
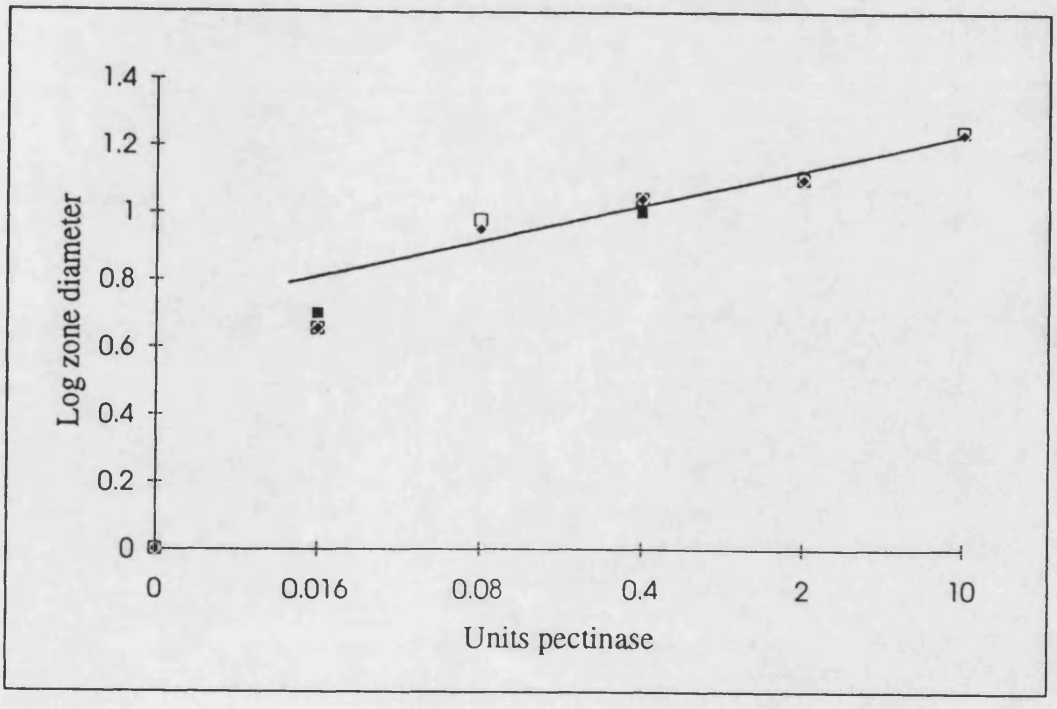


Figure 22: A scatter plot with line of best fit of commercial pectinase activity by cup-plate assay.



4.2.11.4. Comparison of PG and PL enzyme production by seven isolates of *F.o.e.*

This experiment was conducted to compare the seven isolates ranging from highly aggressive to non-pathogenic with respect to the production of PG and PL. The isolates were grown on cell walls to induce enzyme production under controlled conditions. Sampling was done at optimal time as previously established (72 and 144 h for PG and PL respectively). Growth characteristics and pH for each isolate were recorded as a precaution against any significant differences between isolates, which might affect enzyme production and assessment.

Figure 23: PG production from the seven *F.o.e.* isolates sampled after 72 hours on cell wall medium.

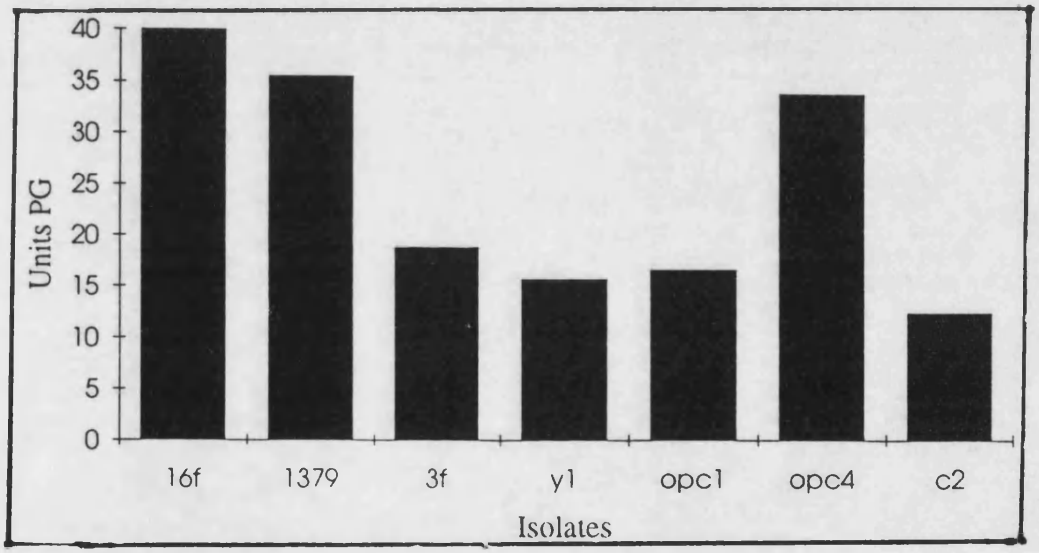
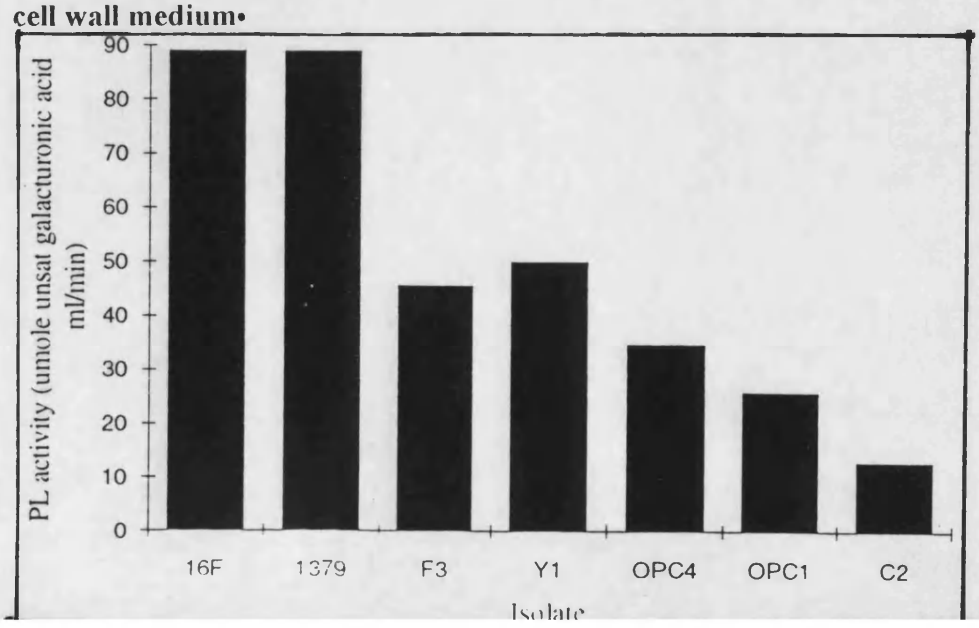


Figure 24: PL Production from the seven *F.o.e.* isolates sampled after 144 hours on cell wall medium.



All isolates produced PG and PL. PG activity ranged from 13.4 - 40 units and PL 13.7 - 89.9 units (Figs. 23 and 24). Thus, production of PG by 16F and 1379 was ≥200% greater than four other isolates and PL was ≥200% above all five other isolates. These levels of activity coincided with the most highly aggressive isolates (16F and 1379) but with OPC_4 PG activity was also high. In comparison, the non-pathogenic isolate produced the lowest activity of both enzymes. Growth and pH curves were also similar thus the data for enzyme activity at sampling times were comparable. Growth of the isolates ranged between 0.25×10^7 - 0.44×10^7 spores per ml at 72 h and 4×10^7 - 6.8×10^7 spores per ml at 72 h and 4×10^7 - 6.8×10^7 spores per ml at 144 h (Figs. 25 and 26). The pH ranged from 7.3 - 7.5 at 72 h and 7.59 - 7.8 at 144 h (Figs. 27 and 28). These small differences are unlikely to explain the substantial differences in enzyme production between isolates.

Fig. 25: Growth of the seven isolates after 72 h on cell wall medium.

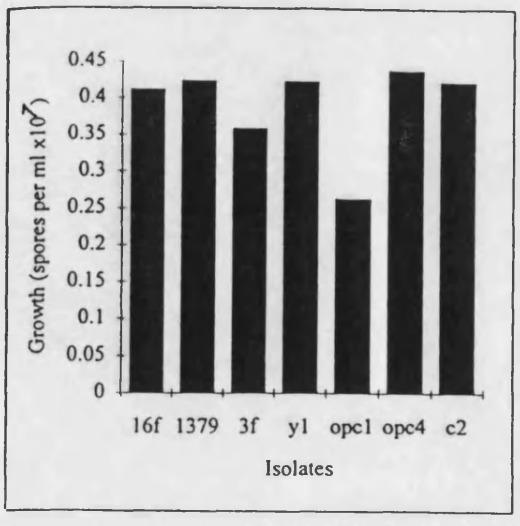


Fig. 26:Growth of the seven isolates after 144 h on medium.

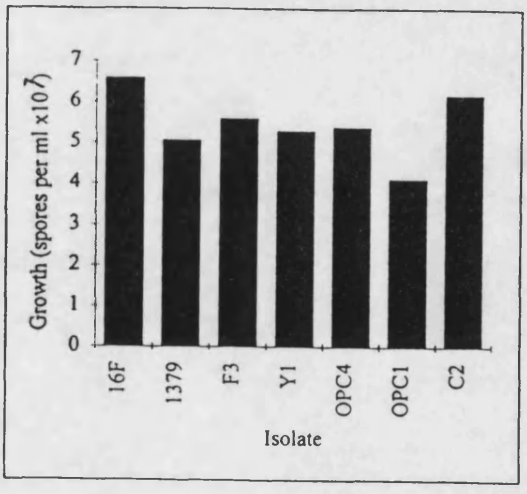


Fig. 27: pH of culture fluid of seven isolates after 72 h

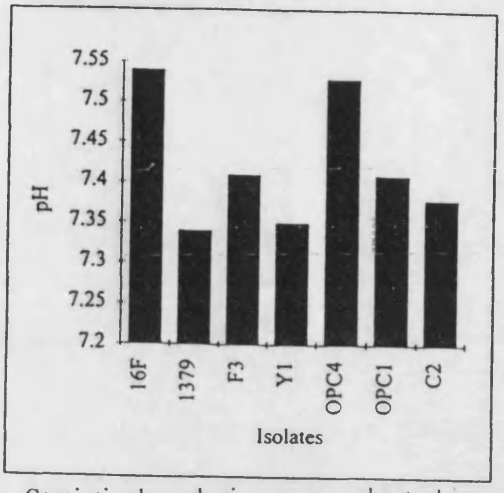
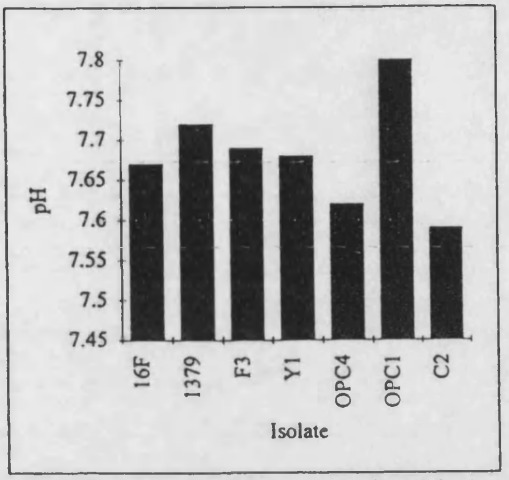
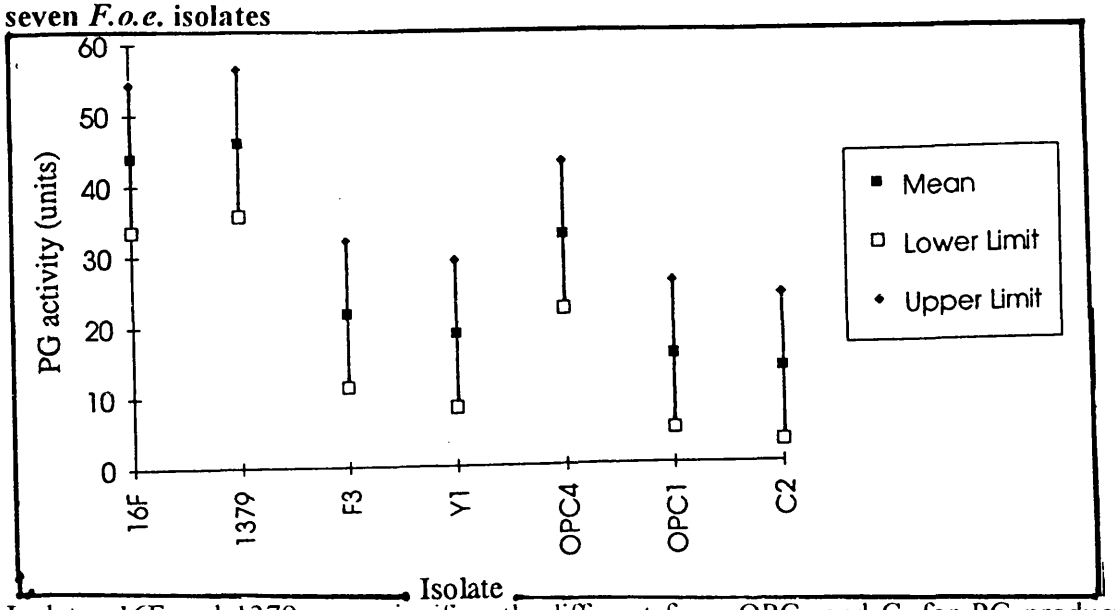


Fig. 28: pH of culture fluid of seven isolates after 144 h



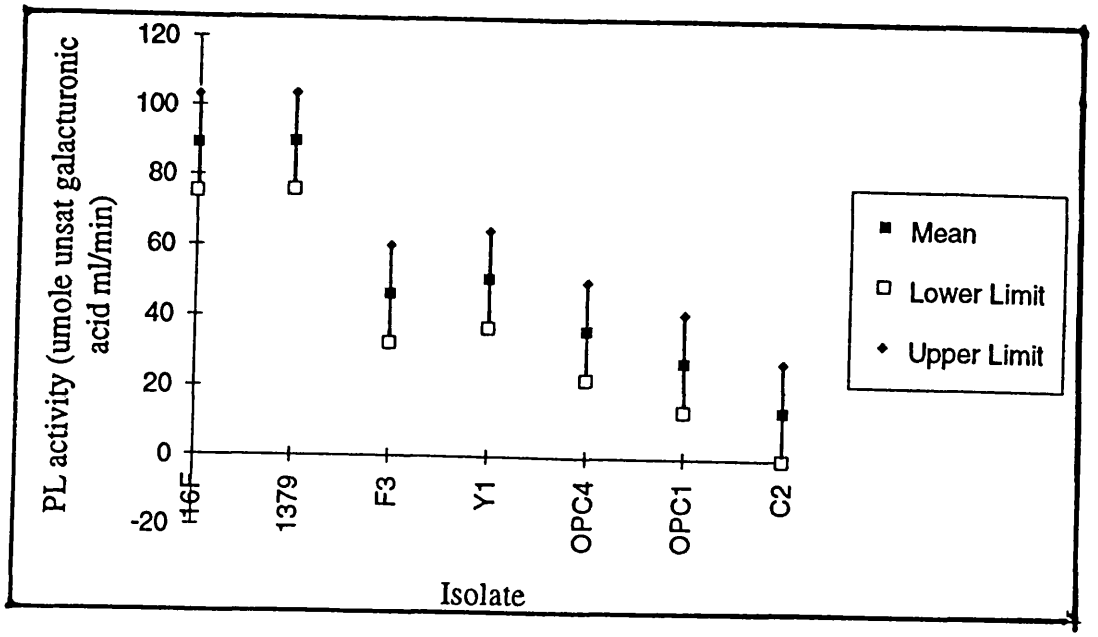
Statistical analysis was conducted in order to establish any significant relationship between pathogenicity and enzyme production. From the data obtained for the seven isolates it was, therefore, necessary to determine whether the differences seen in PG and PL production on the days of sampling could have arisen by error variation instead of being related to pathogenicity of the isolates. An analysis of variance test (ANOVA) tested the data for variance.

Figure 29a: A graphical representation of significance data for PG production from seven *F.o.e.* isolates



Isolates 16F and 1379 were significantly different from OPC_1 and C_2 for PG production and 1379 was significantly different from F_3 and Y_1 (Fig. 29a). Anova tests conducted for production of PL from the seven isolates showed that, isolates 16F and 1379 were significantly different from all the other isolates. However, F_3 and Y_1 were significant only from isolate C_2 (Fig. 29 b).

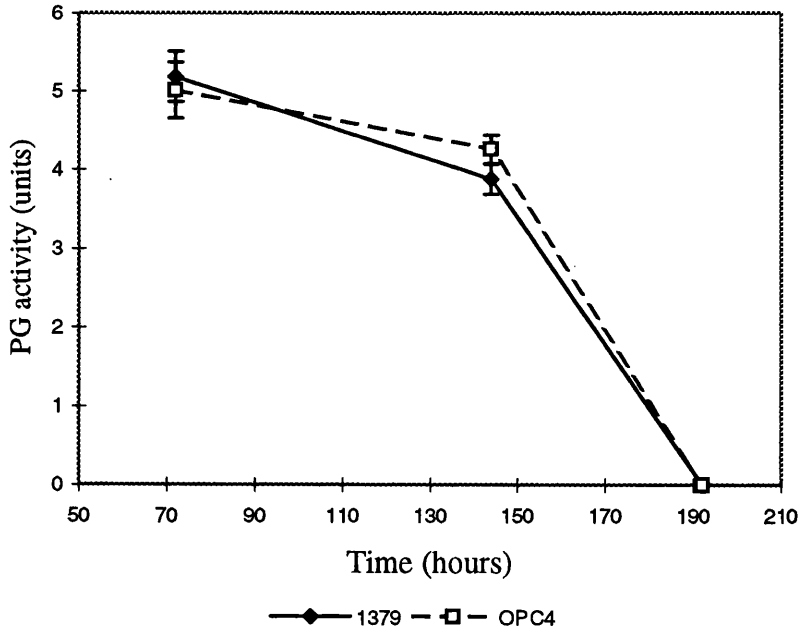
Figure 29b: A graphical representation of significance data for PL production from seven *F.o.e.* isolates



4.2.11.5 Enzyme detection in *F.o.e.* (isolates 1379 and OPC_4) infused petioles of a susceptible clone.

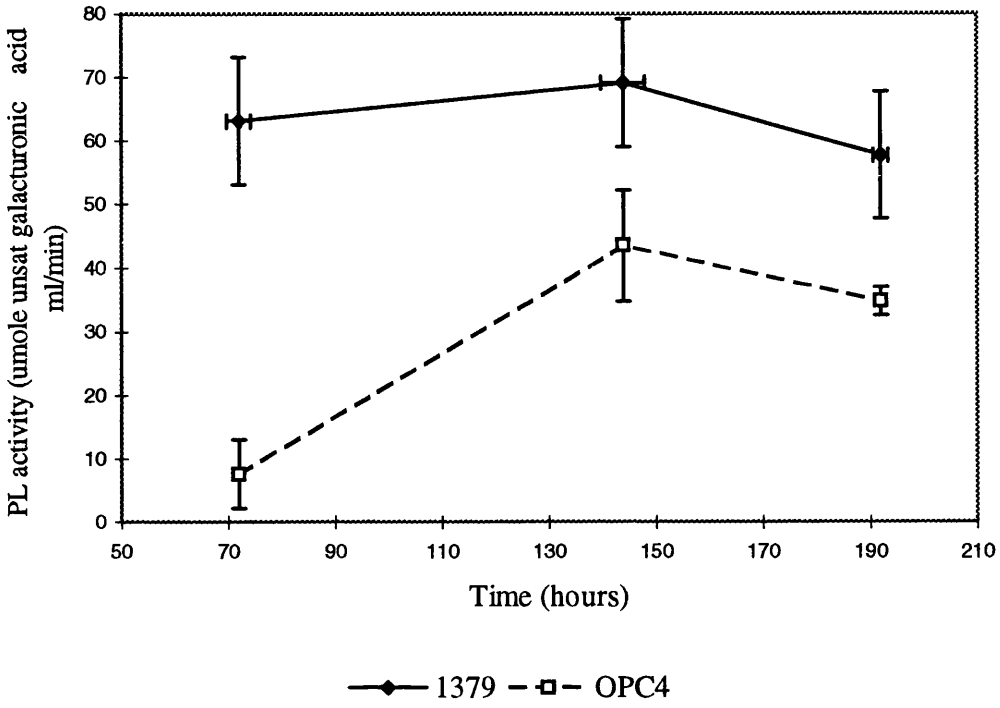
To examine PG and PL production *in vivo* petioles from a susceptible clone (UF177) were inoculated with a highly aggressive isolate (1379) and a less aggressive isolate (OPC_4). The petioles were incubated for 72, 144 and 192 h. Tissue was extracted for enzymes at each interval and extracts were assayed for PG and PL activity. Low PG activity was detected for both isolates after 72 h but decreased and by 192 h was no longer detectable (Fig. 30). PL activity for both isolates was also detected at 72 h but this increased up to 144 h and only slightly decreased by 192 h. The highly aggressive isolate (1379) produced PL at a much faster rate than the less aggressive (OPC_4) isolate; thus, at 72 h PL activity was eight fold greater in petioles infused with 1379 (Fig. 31).

Figure 30: PG production in petioles inoculated with isolates 1379 and OPC_4.



Values are the mean (±SE) of three replicates.

Figure 31: PL Production in petioles inoculated with isolates 1379 and OPC_4.



Values are the mean (±SE) of two replicates.

4.2.11.6 Detection of PL activity from *F.o.e.* - infused petioles of resistant and susceptible clones.

The previous study showed a faster rate of PL production by a more aggressive isolate in petioles of a susceptible oil palm clone. It remained to examine the rate of PL production in petioles of both susceptible and resistant clones infused with isolate F_3. Petioles infused with F_3 were incubated for 1-4 d and at each interval, tissue was extracted for PL activity. PL activity was detected in both clones. Activity in the susceptible clone was detected at very low levels (0.1 units) by 2 d but reached highest activity (5 units) by 3 d then rapidly declined and after 4 d activity again at the lowest was detectable level (0.1) (Table 22). PL activity was significantly higher in the resistant clone at 2d (1.25 units) then rapidly

decreased and by 4d was no longer detectable (Table 22); thus at 3d PL activity was *ca.* 8 fold greater in the susceptible clone compared to resistant clone.

Table 22: PL activities* in extracts of F.o.e. infused WR and WS oil palm petioles.

	DAYS AFTER INOCULATION			
CLONE	1	2	3	4
UF28 (WR)	0 a	1.25 b	0.6 a	0 a
UF177 (WS)	0 a	0.1 a	5.0 b	0.1 a

Activities in units, μmole unsaturated galacturonic acid released ml^{-1} h^{-1}.

Within each column, figures with the same letter are not significantly different ($P>0.05$) using Kruskal Wallis and repeated Mann Whitney tests.

To summarize, PAL activity was significantly higher in a resistant clone (inoculated and uninoculated) than in a susceptible clone throughout the experiment with no increase in either clone , 4 d after inoculation. Low and similar peroxidase activities were detected in both inoculated and uninoculated petioles, 2 and 4 d, after inoculation and there was no significant difference in the overall levels between resistant and susceptible clones. Both glucanase and chitinase were detected in all the samples tested but no significant differences in levels of activity was evident between resistant and susceptible clones throughout the experiment. The most aggressive isolates (16F and 1379) and also OPC_4 produced significantly higher levels of PG and PL *in vitro* on host cell walls as sole source of carbon compared to the other five isolates and the non-pathogenic isolate (C_2) produced the lowest activity. *In vivo*, the rate of production of PL was faster with a highly

aggressive isolate than by a moderate aggressive isolate and the level of activity was significantly higher at day 3 in a susceptible than a resistant clone.

4.3 DISCUSSION.

This section was designed to investigate if resistance or susceptibility was expressed in oil palm roots or petioles with the aim of developing a model system which may be used as a rapid screen for resistance and to study the mechanisms of resistance involved in *Fusarium* wilt of oil palm.

Facile model systems are often required in the study of plant diseases especially for study of cell-cell interactions in chronic vascular diseases. Several authors have reported that resistance to vascular wilts is expressed in leaves or stems, for example, with *Verticillium* (Bell, 1967; Tjamos and Smith, 1974; Flood *et al.,* 1978; Flood and Milton, 1982) and with *Fusarium* (Hutson and Smith, 1980; Marley and Hillocks, 1993). However, the swollen pseudostem base 'bulb' of oil palm is difficult to infuse and examine because of its complex vascular architecture and thus, for the first time, oil palm petioles and roots were tested for their ability to express resistance following infusion with conidia.

In developing this technique, a modification of the method of Newcombe *et al.* (1989) was used whereby conidia were infused directly into the petiole or root xylem. The method of Beckman *et al.* (1962) was also adopted where inert particles were used to identify 'trap sites' in the xylem. These 'trap sites' occur where the water flow transfers from one vessel to another or at the end of vessels. These sites are important in terms of resistance because conidia become impacted, germinate and grow through pits into the adjacent vessels in

order to continue colonization. Thus, at this stage the host may have time to respond and if this response is significantly rapid then further invasion may be prevented.

In this study, primary colonization by the fungus at 'trap sites' in oil palm roots clearly correlated with the wilt reaction of four oil palm clones ranging from extremely wilt susceptible to wilt resistant i.e. least colonization occurred in resistant material. Newcombe *et al.* (1989) similarly reported that differential colonization of the xylem by *Verticillium* occurred in stem cuttings of alfalfa clones. Also, in root xylem of resistant tomato varieties infected with *Verticillium* or *Fusarium,* Hutson and Smith (1980) found that the pathogens were significantly restricted to the xylem of lower nodes 14 d after inoculation but colonization in susceptible plants continued to increase for 28 d. The colonization of roots of resistant palm clones by *F.o.e.* was restricted to the lower vessels extending from the cut ends and from here the number of infected vessels rapidly declined with increasing distance. This observation is supported by results of Harrison and Beckman (1982) who found that colonization by *V. dahliae* and *F. oxysporum* f. sp. *vasinfectum* was restricted to the lower portions of resistant cotton plants.

In the current study, lateral vessel to vessel invasion through intertracheary pits and ramification of vascular tissue by *F.o.e.* hyphae was a common feature of infiltrated roots from susceptible oil palm clones. Similar vessel to vessel colonization was observed in elm xylem tissue (MacDonald and MacNabb, 1970). In contrast, Bishop and Cooper (1983) found that in vascular colonization of susceptible cultivars of tomato by *F. oxysporum* f. sp. *pisi,* vessel to vessel colonization was uncommon but occasionally occurred by penetration of intertracheary pit membranes.

Vessel lengths were investigated in oil palm roots as a possible component of resistance. Beckman *et al.* (1961) found that *Fusarium* microspores required 2-3 d to germinate,

penetrate the end walls and produce spores in the next vessel. Thus, spore trapping at vessel end walls is vital for the success of various resistance responses including production of inhibitors of fungal growth (Beckman *et al.,* 1976; Beckman and Talboys, 1981; Harrison and Beckman, 1982). However, in this study, no difference in oil palm root vessel lengths were detected which could be related to varietal resistance.

Occlusion of vessels by gels is a common feature following infection of both susceptible and resistant varieties of many species by vascular fungi (Vandermolen *et al.,* 1977; Vandermolen, 1978; Mollenhauer, 1976) and may well be a general plant response to stress or wounding. In oil palm roots, xylem parenchyma cells responded to infection by *F.o.e.* or to wounding with the production of gels in the vessel lumens. Gels were detected earlier (2 d after infiltration) and accumulated to a greater extent in resistant than in susceptible clones. Rapid occlusion of vessels by gels appears to represent a first step in the sequence of defence processes (Beckman, 1987). The formation and accumulation of gels have been reported in *Verticillium* - infected alfalfa (Newcombe and Robb, 1988), tomato (Robb *et al.* 1987; Street *et al.*, 1986), carnation (Moreau *et al.,* 1978), *Fusarium*-infected pea (Tessier *et al.*, 1990), celery (Jordan *et al.,* 1988), cotton (Bugbee, 1970; Shi *et al.,* 1992) and in banana (Beckman *et al.,* 1962). Gels have also been shown as plugs above spore trapping sites in infected vessels where they persist for several days in resistant reactions and appear resistant to physical or chemical degradation (Beckman and Zarogian, 1967). Also, at this point they cut off the transpiration stream and serve to embed and immobilize the secondary spores of the pathogen at the site of their formation just above the trapping sites thus preventing any further colonization. However, in susceptible reactions, these gels appear to be weakened and shear under transpiration tension (Beckman and Keller, 1977).

In the present study there was a positive correlation between resistance and vascular occlusion by gels in oil palm. Gels were rapidly formed in advance of pathogen spread and colonization declined with distance from the site of infiltration in resistant palms. This result is in agreement with studies conducted on resistant tomato varieties infected with *F. oxysporum* f. sp. *lycopersici* (Beckman *et al.*, 1972). In contrast, Mace (1978) observed a sparse and delayed gel production and occlusion of vessels in a *Verticillium* infected resistant cotton cultivar. He concluded that gelation is not a significant factor in resistance to *Verticillium* wilt in cotton.

The occlusion of vessels with tyloses was also evident in roots of resistant palm clones 8 d after infiltration with *F.o.e.* conidia. Production of tyloses following infection by wilt pathogens have been reported in *Verticillium* infected tomato (Dixon and Pegg, 1969; Sinha and Wood 1968; Hutson and Smith, 1980), sunflower (Robb *et al.*, 1979 a), hop (Talboys, 1958 b), cotton (Mace, 1978), *Fusarium* infected tomato (Beckman *et al.*, 1972; Hutson and Smith, 1980), cotton (Shi *et al.*, 1992) and in banana (Beckman *et al.*, 1962). In this current study, occlusion of vessels with tyloses always occurred above trapping sites subsequent to gel formation (later than 4 d after inoculation). There were significantly more tyloses produced in roots from resistant clones than susceptible and they coincided with restriction of upward spread of *F.o.e.* Similar results for colonization and tylosis were reported by Tjamos and Smith (1975) for a resistant tomato cultivar inoculated with *V. albo-atrum* but Talboys (1958 b) working on Verticillium wilt of hop and strawberry, found an inverse relationship between that mycelial vascular colonization and tylosis.

Thus, the occlusion of vessels with tyloses above infection and trapping sites following gel production and subsequent restriction of upward spread of *F.o.e.* in roots from resistant

palms provides circumstantial evidence for the involvement of these physical barriers in resistance of oil palm. The timing of tylosis in relation to vessel occlusion was extensively discussed by Beckman *et al.* (1962) in relation to *F. oxysporum* f. sp. *cubense* infected banana. It was suggested that gelation must occur before and persist after resporulation; but tyloses must occlude the vessels before gel disappearance and be extensive enough to contain the fungus which has advanced and resporulated within the gel.

Although resistance and susceptibility were expressed in oil palm roots using the infused, attached, severed root technique and a good correlation of these results with the nursery trials was obtained, the technique had several limitations. The process of severing roots under water (to prevent air ingression) is time consuming and destructive; cutting several roots for infusion causes plant death. Contamination of root xylem with bacteria was very frequent especially when the plants had to be incubated for 14 d. Microscopic assessment is often subjective especially the quantification of growing hyphae in xylem vessels. The scattered nature of vascular bundles in oil palm made it difficult to determine secondary colonization in the adjacent vessels. However, on very rare occasions where adjacent vessels existed, extensive secondary colonization occurred in susceptible plants whilst no secondary colonization was observed in resistant clones. Consequently, an alternative to the infused severed root technique was sought and thus infused oil palm petioles were tried.

Infusion of petioles from three resistant and three susceptible clones with *F.o.e.* distinguished resistance and susceptibility within 8 d; the susceptible clone became necrotic whilst the resistant clone remained green and intact (Mepsted *et al.*, 1995). Furthermore, extensive mycelial growth developed in susceptible petioles but growth was very limited in

resistant clones. Petiole vessel lengths were investigated but as reported before in roots, no significant differences in petiole vessel length were evident that could be related to varietal resistance.

Some antifungal substances occur in plants as preformed compounds such as saponins and phenolics (Mansfield, 1983). It was evident that xylem fluid extracted from petioles of a resistant clone, at time zero, contained preformed antifungal substances. This observation concurred with the work conducted by Assef *et al.* (1986) who found preformed antifungal compounds in root extracts of date palm resistant to *F. oxysporum* f. sp. *albedinis*. These substances were extracted from susceptible date palm cultivars while in this work, although some preformed antifungal substance(s) were detected in extracts from comminuted tissue of both resistant and susceptible clones, there was no significant varietal difference that could explain the reduction of the amount of *F.o.e* in the WR clone. However, induced antifungal substances were also produced by oil palm petioles. Antifungal compound(s) detected in this study are regarded as phytoalexins and the term 'phytoalexin' is used here in its broad sense (i.e. antifungal substances reaching inhibitory levels after infection or wounding). Antifungal compound(s) accumulated in xylem fluid and in tissue of resistant oil palm petioles in response to infusion with *F.o.e.;* greatest accumulation (based on bioassay) occurred when the whole petiole was infused so that intercellular spaces as well as petiole xylem were challenged with *F.o.e.* conidia and this was followed by extraction from the entire petiole. Antifungal compounds were not detected in any of the susceptible clones tested in this study and stimulation of germination frequently occurred following extraction from these clones. There have been many reports in which phytoalexins have been implicated in resistance and unlike the results obtained here, most workers have detected phytoalexin accumulation in both resistant and

susceptible lines; resistance responses were characterized by more rapid accumulation to higher levels e.g. in *Verticillium* infected resistant tomato (Tjamos and Smith, 1974), cotton (Bell, 1969; Mace, 1978; Zhang *et al.;* Shi *et al.,* 1993), in Fusarium infected tomato (Hammerschlag and Mace, 1975; Stromberg and Corden, 1977) and in *F.o.e.*-infected oil palm roots (Taquet *et al.,* 1985; Vernenghi *et al.,* 1987; Diabate *et al.,* 1990). These latter authors detected seven phenolic compounds which were mainly benzoic and cinnamic acids derivatives. Vernenghi *et al.* (1987) also claimed to have identified inhibitory compounds from oil palm roots and described them as oxygenated derivatives of polyenic fatty acids. Attempts were made during this study to separate and identify the antifungal compounds involved using the solvent systems of the above authors, but no inhibitory compounds were detected using U.V. reaction to spray reagents or fungal bioassays. One reason for this failure of detection may be because the compounds detected here were different from those in published work. For example, in this study the inhibitory compounds were not soluble in methanol whilst those compounds detected by Taquet *et al.* (1985) and Vernenghi *et al.* (1987) were methanol soluble. Solvent systems were used in conjunction with t.l.c but no inhibition zones were detected. The failure to detect zones of inhibition on the plate using extracts which previously showed antifungal activity on slides, may have been due to oxidation following drying of compounds as monolayers on to silica plates or tight adsorption on to the silica (M. Beale pers. comm.). Future work should employ silica columns where the extracts can be collected without drying as occurs with thin layer chromatography. This latter method has been demonstrated to be very effective in the study of antifungal compounds in *Theobrom cacao* (Resende, 1994).

The non pathogenic isolate, C2, was inhibited at lower levels of extracts than the other isolates but these other isolates were inhibited at similar concentrations irrespective of

their comparative aggressiveness. Thus, aggressiveness was probably not linked to phytoalexin tolerance or ability to degrade the inhibitory substances. Cruickshank and Perrin (1963 b) similarly demonstrated that on pea pods, *Septoria pisi* (an aggressive pea pathogen) and *Ascochyta pisi* (less aggressive) were both sensitive to pisatin.

An effective disease screening procedure should be simple, rapid and clearly differentiate between susceptible and resistant genotypes (Durbin, 1981). Thus, using assessment of external symptoms (browning score) then the petiole infusion technique may be used as a rapid (8 d) alternative to conventional disease screening at the nursery stage which takes 8 - 14 months. A similar approach has been suggested for Verticillium wilt of lucerne (Yu *et al.*, 1993). These workers observed chlorosis of infected susceptible alfalfa stem cuttings 7 d after infusion with spore suspension of *V. albo-atrum.* They also suggested that the technique could be used in breeding programmes to screen rapidly large numbers of plants as an alternative to conventional screening procedures and the current work would suggest that the technique could be used to screen large numbers of oil palm progenies.

In the oil palm - *F.o.e.* interaction, this technique could also be of considerable value to the selection of resistant individual palms in the plantation. Currently, oil palms are selected in the field on the basis of absence of external symptoms although these could be 'escapes' or merely tolerant to disease; also low levels of infection can be present in symptomless palms (Mepsted.,1992). Thus the current criterion used, selection from progenies with a lower than average incidence of wilt symptoms, does not guarantee the resistance of the individual palm. Using the petiole infiltration technique it should be possible to assess individual, high yielding palms for resistance in the field. This approach is currently being tested in commercial plantations in Ivory Coast and Zaire (Mepsted, pers. comm.).

The phenylpropanoid biosynthetic pathway is the key synthetic route for many phenolic compounds associated with defence such as lignin and certain phytoalexins (Jones, 1984). The key enzyme in this pathway, phenylalanine ammonia-lyase (PAL) has been shown to be induced by pathogens or elicitors (e.g. pathogen cell wall components) in many plant species. Thus, the induction of this enzyme may be linked to the defence responses triggered during host-pathogen interactions and may be an indicator of defence activation. In order for a pathogen not to induce these defence responses it must have some method of avoiding recognition, or of suppressing the activation mechanism.

The level of PAL activity was higher in an inoculated, wounded resistant clone than in the susceptible clone and remained so throughout the experiment but there was no significant difference in activity that could be linked with resistance. This work is supported by results obtained by Corsini and Pavek (1980) who found that there was no significant difference in PAL activities among four cultivars of *Fusarium* infected tomato studied that could be linked with resistance. However, other workers have been able to demonstrate varietal differences revealed in PAL activity for example, in *Phytophthora* inoculated resistant soybean (Bhattacharyya and Ward, 1988) and in resistant *Eucalyptus* cultivars (Cahill and McComb, 1992).

A slight rise in level of peroxidase (PO) activity was detected only in infected and excised petioles from susceptible clones and polyphenoloxidase (PPO) was not detected in any of the clones tested. These two enzymes are capable of oxidizing phenolic compounds to fungitoxic products and increased PO and PPO activities after infection have been reported in various wilt diseases and have been linked with resistance e.g. *Fusarium* infected tomato (Grzelinska, 1970; Matta and Dimond, 1963; Retig, 1974).

Although glucanase and chitinase activities were detected in all the samples tested, the levels remained similar between resistant and susceptible clones throughout the experiment. These enzymes have the potential to degrade chitin and β-1, 3-glucans in fungal cell walls, since these are major polymeric components of many fungal cell walls the enzymes can be thus inhibitory to fungal growth (Mauch *et al.,* 1988). These workers also showed that the two fungi *Fusarium solani* f.sp. *pisi* and *Fusarium solani* f. sp. *phaseoli*, were similarly strongly inhibited by these enzymes, indicating that the differential pathogenicity of the two fungi is not due to the differential sensitivity to the wall degrading enzymes from pea.

It has been demonstrated in this study (e.g. nursery trials, inoculation of roots and petioles) that more extensive colonization by *F.o.e.* characterizes aggressive isolates in a susceptible host. What has not been fully understood, though, is whether this is due to a greater inherent growth rate, more effective pathogenicity factors, superior ability to cope with host defenses, lack of recognition by the host to respond on time or a combination of these. In an attempt to investigate putative pathogenicity factors involved in this host-pathogen interaction pectic enzymes produced by the pathogen were studied. In the process of colonization, it has been observed that, the pathogen must encounter and penetrate numerous cell walls as it colonizes the host during root penetration, through pits at vessel end walls and between adjacent vessels (Cooper, 1983). Cell wall degrading enzymes (CWDE) are presumably important in breaching these barriers and in the subsequent release of nutrients necessary to provide vascular pathogens with carbon sources (Bateman and Millar, 1966; Bateman and Basham, 1973). Another likely mechanism where pectic enzymes could contribute to aggressiveness of *F.o.e.* is the degradation of gels; Beckman (1964) postulated that in *Fusarium* infected banana, a high

level of pectolytic activity could result in dissolution of gels and hence the release of the pathogen before the gel becomes infused with inhibitors or is made resistant to enzymic attack. Widespread vascular gelation and tylosis have been demonstrated in this host pathogen interaction and Beckman's hypothesis may also be appropriate to vascular wilt of oil palm. It also seems likely that the browning reactions characteristic of necrosis and the softening of the infused petiole tissue of the susceptible clone 7 - 8 d after infiltration with aggressive *F.o.e.* isolates could be due to the involvement of pectic enzymes. The ability of pectic enzymes to macerate plant tissue and kill protoplasts has been emphasized as an important step involved in pathogenesis (Cooper, 1983; Basham and Bateman, 1975) .The pH of an environment in which any pathogen grows has a marked influence upon the stability of the enzymes produced and on their production (Cooper, 1976). Many studies have revealed that polygalacturonase (PG) shows highest activity at acidic pH (4 - 5.5) while pectin lyase (PL) requires an alkaline pH (7.5 - 8.5) (Cooper *et al.* , 1978; Fernadez *et al.*, 1993; Trique, 1971; Osagie and Obuekwe, 1991). Also, analysis of constituents of vascular fluid may enable an artificial substitute to be formulated (Hignett *et al.*, 1984). Thus, growth on various inducing substrates to determine the inherent enzyme synthetic capability of each isolate and the effects of pH were investigated.

In this study, the most aggressive isolates consistently produced significantly higher levels of PG and PL *in vitro*. This result and that obtained by Carder *et al.* (1987) on hop infected with *Verticillium dahliae* show that pectic enzymes production of different wilt pathogens could be linked to aggressiveness. The role of pectic enzymes in aggressiveness was further demonstrated by Durrands and Cooper (1988) using pectinase-deficient mutants of *Verticillium albo-atrum.* Also, production of PL *in vivo* was faster by aggressive isolates of *F.o.e.* indicating their potential to degrade host physical barriers

including gels and pit membranes. Deese and Stahmann (1962) also reported a rapid pectic enzyme production in stems of tomato plants infected with *F. oxysporum* f. sp. *lycopersici.*

In conclusion, breeding for resistance is the only practical, long term control for Fusarium wilt of oil palm. Breeding programmes have been constrained in the past partly due to a lack of a rapid, reproducible screen for resistance and also due to the lack of understanding of the inheritance of resistance and the underlying resistance mechanisms. The development of a rapid screen for resistance using infused petioles as described in this thesis plus the future applications of the technique in the field and the laboratory are likely to make significant contributions to understanding resistance of oil palm to Fusarium wilt.

4.6 FUTURE RESEARCH.

This work has revealed some exciting results which lay firm foundations for future research in some aspects of this interesting host pathogen interaction and application of the technique as a 'model' system to study this complex disease of oil palm.

There was some evidence that variations in virulence occurred with some isolates when inoculated on some oil palm material but overall this was not the case; no genetic interaction was proven. This work should be extended to include a wider range of plant and fungal material and to increase replication. To avoid introducing very aggressive or differentially virulent strains into new areas, these studies should be conducted in a country which does not grow oil palm commercially under standard environmental conditions, but space in a controlled growth facility may be a limiting factor as is the supply of specific materials.

Soil isolates of *F. oxysporum* do not induce wilt symptoms but rather promote growth to significantly high levels in some instances. In the light of the absence of vascular wilt disease in Malaysia, it is worth investigating whether avirulent soil isolates can suppress the development of vascular wilt of oil palm with a view to a biological control method in the future.

Oil and date palms occasionally overlap in some African countries including Nigeria, Burkina Faso, Chad and Sudan. Preliminary work here has indicated that cross infection by form species adapted to palms can occur. This phenomenon needs to be studied further

using wider range of palm genotypes and *F. oxysporum* form species before definite conclusions can be made.

In vitro screening for resistance would be an advantage over the conventional method which takes up to 7-8 months to complete but results obtained here using callus material supplied by Unifield were not encouraging. The possibility of influencing expression of resistance/susceptibility by the manipulation of hormones at the initiation of callus from the parent tissue could be further explored.

However,this investigation clearly demonstrated that resistance or susceptibility is expressed in oil palm petioles and roots eight days following infusion with *F.o.e.* conidia under controlled conditions. These responses correlated with disease resistance and susceptibility of whole plants as determined by conventional pathogenicity test in which symptoms are apparent 7-8 months after inoculation. Currently, field palms are selected on the basis of no external symptoms and from progenies with a lower than average incidence of wilt, a technique which does not guarantee the resistance of the individual palms. With the petiole infusion technique, and possible improvement, it will be possible to assess individual, high yielding palms for resistance in the field and this work is currently being undertaken in Ivory Coast. Data on the resistance of individual palms instead of observations based on lack of external symptoms in a field palm should provide relaible assessment of disease reaction and facilitate a better understanding of the genetics of wilt resistance.

Investigation of the basis for resistance revealed that vessel lengths in roots and petioles could not be linked with resistance, but it was observed in susceptible and not resistant clones, that root vessels extended into the pseudobulb. Continuity of vessels at the root / bulb interface (*ca.* 3 cm below the bulb) should be further investigated.

Rapid xylem occlusion by gels and tyloses were associated with resistance and antifungal substance(s) also rapidly accumulated in the resistant material. However, identification of these compound(s) which would facilitate quantification (e.g. by physicochemical assay) leading to evaluation of their role in resistance was not achieved. The failure to detect zones of inhibition on t.l.c. plates which had previously shown antifungal activity using slide bioassays may have been due to oxidation following drying onto silica plates or adsorption to the silica. Thus future work should employ silica columns whereby the extracts would not be dried as a thin layer onto silica. Also, to minimize possible oxidation, extracts should be dried and stored under nitrogen.

Very little is known about pathogenicity factors from *F.o.e.* such as CWDE or toxins. However, some evidence from preliminary experiments has indicated the involvement of fusaric acid (toxin) in pathogenicity of *F.o.e.* to oil palm and the use of toxins for *in vitro* selection for novel resistance has been investigated (Cooper *et al.*,1989). In the current study, rate and extent of gel formation were implicated in resistance while production of pectic enzymes was correlated with degree of aggressiveness of the *Fusarium* isolates. It is likely that the function of these pectic enzymes would be to suppress or overcome host defences such as the degradation of gels in xylem vessels. Future reseach on these enzymes should employ the use of *F.o.e.* mutants (obtained by site directed mutagenesis) to enable the understanding of their role in pathogenesis.

REFERENCES

Aderungboye, F.O. (1981). Significance of vascular wilt in oil palm plantations in Nigeria. In: *The Oil Palm in Agriculture in the Eighties.* Eds. Pushparajah, E. and Chew Pop Sew. Kuala Lumpar: Incorporate Society of Planters, pp. 1-11.

Alexander, L.J. and Tucker, C.M. (1945).
Physiologic specialization in the tomato wilt fungus.
Fusarium oxysporum f. sp. *lycopersici. Journal of Agricultural Research.*
70: 303-313.

Alexander, L.J. (1962). Susceptibility of certain Verticillium resistant tomato varieties to an Ohio isolate of the pathogen. *Phytopathology* **52:** 998-1,000.

Anon. (1951). Jaarverslag over 1950. Department of Agriculture. Surinam. 1950 p 87. In: *Review of Applied Mycology* **32:** 546-547 (abstract).

Anon. (1960). Annual Report of the Cameroon Development Corporation. 1959. p 29. In: *Review of Applied Mycology* **40:** 148 (abstract).

Anon. (1991). Oil palm development in Nigeria: Status and prospects. In: Anon, (1991). The Role of Research Networks for the Development of oil palm in Africa. AFOPDA/BUROTROP seminar. Abidjan. June 1991. Paris. BUROTROP

Artes, E.P. and **Tena, A.** (1990). Purification and characterization of pectic enzymes from two races of *Fusarium oxysporum* f. sp. *ciceri* differing in virulence to chickpea (*Cicerarietinum L*). *Physiological and Molecular Plant Pathology,* **37:** 107-124.

Assef, G.M., Assari, K. and **Vincent, E.J.** (1986). Occurrence of an antifungal principle in root extract of a Bayoud-resistant date palm cultivar. *Netherlands Journal of Plant Pathology,* **92:** 43-47.

Bachy, A. (1970). La fusariose du palmier a huile. *Oléagineux,* **24:** 265-267.

Bailey, J.A. and **Deverall, B.J.** (1971). Formation and activity of phaseollin in the interaction between bean hypocotyls *(Phaseolus vulgaris)* and physiological races of *Colletotrichum lindemuthianum. Physiological Plant Pathology,* **1:** 435-449.

Bailey, J.A. (1982). Mechanisms of phytoalexin accumulation. In *Phytoalexins* (eds. J.A. Bailey and J.W. Mansfield), Blackie, Glasgow, pp. 289-231.

Banfield, W.M. (1941). Distribution by the sap stream of spores of three fungi that induce vascular wilt diseases in the elm. *Journal of Agricultural Research,* **62:** 637-681.
Banfield, W.M. (1968). Dutch elm disease recccurrance and recovery in American elm. *Phytopathology Zeitschrift,* **62:** 21 - 60.

Bateman, D.F. (1963). The 'macerating enzyme' of *Rhizoctonia solani. Phytopathology*, **53:** 1178-1186.

Basham, H.G. and **Bateman, D.F.** (1975). Killing of plant cells by pectic enzymes : the lack of direct injurious interaction between pectic enzymes or their soluble reaction products and plant cells. *Phytopathology,* **65 :** 114-153.

Bateman, D.F. (1964). An induced mechanism of tissue resistance to polygalacturonase in *Rhizoctonia*-infected hypocotyles of bean. *Phytopathology,* **54 :** 438-445.

Bateman, D.F. (1976). Plant cell wall hydrolysis by pathogens. In *Biochemical aspects of plant parasite relationships,* Ed. by J. Friend and D.R. Threlfall pp 79-103. Academic press, London, New York and San Francisco.

Bateman, D.F. and **Millar, R.L.** (1966). Pectic enzymes in tissue degradation. Annual Review of *Phytopathology,* **4:** 119-146.

Bateman, D.F. and **Basham, H.G.** (1973). Degradation of plant cell walls and membranes by microbial enzymes. *Physiological Plant Pathology*, **4:** 316-355.

Bateman, D.F. and **Basham, H.G.** (1976). Degradation of plant cell walls and membranes by microbial enzymes. In *Encyclopaedia of plant physiology,* **1,** *Physiological Plant Pathology.* Ed. by Heitefuss and P.H. Williams, pp 316-355 Springer Verlag.

Beckman, C.H. (1964). Host responses to vascular infection. *Annual Review of Pytopathology*, **2:** 231-252.

Beckman*h*, C.H. (1966). Cell irritability and the localization of vascular infection in plants. *Phytopathology,* **56:** 821-824.

Beckman, C.H. (1967). Respiratory response of radish varieties resistant and susceptible to vascular infection by *Fusarium oxysporum* f. sp. *conglutinans. Phytopathology,* **57:** 699-702.

Beckman, C.H. (1969 a). The mechanics of gel formation by swelling of simulated plant cell wall membranes and perforation plates of banana root vessels. *Phytopathology,* **59:** 837-843

Beckman, C.H. (1969 b). Plasticizing of walls and gel induction in banana root vessels infected with *Fusarium oxysporum. Phytopatholgy,* **59:** 477-483.

Beckman, C.H. (1971). The plasticizing of plant cell walls and tylose formation - a model. *Physiological Plant Pathology,* **1:** 1-10.

Beckman, C.H. (1987). *The Nature of Wilt Diseases of Plants*: American Phytopathological Society.

Beckman, C.H. and **Zaroogian, G.H.** (1967). Origin and composition of vascular gel in infected banana roots. *Phytopathology*, **57:** 11-13.

Beckman, C.H. Elgersma, D.M. and **Machardy, W.E.** (1972). The localization of fusarial infections in the vascular tissue of single dominant-gene resistant tomatoes. *Phytopathology*, **62:** 1256-1260.

Beckman, C.H. Vendermolen, E.G. , Muller, W.C. and **Mace, M.E.** (1976). Vascular structure and distribution of vascular pathogens in cotton. *Physiological Plant Pathology*, **9:** 87-94.

Beckman, C.H. and **Talboys, P.W.** (1981). Anatomy of Resistance. In *Fungal Wilt Diseases of Plants.* Ed. M.E. Mace, A.A, Bell and C.H. Beckman. pp 487-521.

Beckman, C.H., Vandermolen, E.G., Muller, W.C. and **Mace, M.E.** (1976). Vascular structure and distribution of vascular pathogens in cotton. *Physiological Plant Pathology*, **9:** 87-94.

Beckman, C.H., Halmos, S. and **Mace, M.E.** (1962). The interaction of host, pathogen and temperature in relation to susceptibility to *Fusarium* wilt in banana. *Phytopathology*, **52:** 134-140.

Beckman, C.H. and **Keller, J.L.** (1977). Vessels do end! *Phytopathology*, **67:** 954-956.

Beckman, C.H. Mace, M.E., Holmos, S. and **McGaham, M.W.** (1961). Physical barriers associated with resistance in *Fusarium* wilt of banana. *Phytopathology*, **51:** 507-518.

Beckman, C.H., Muller, W.C. and **Mace, M.E.** (1974). The stabilization of artificial and natural cell wall membranes by phenolic infusion and its relation to wilt disease resistance. *Phytopathology* **64:** 1214-1220.

Behnke, M. (1979). Selection of potato callus for resistance to culture filtrates of *Phytophthora infestans* and regeneration of resistant plants. *Theoretical and Applied Genetics.*, **55:** 69-71.

Bell, A.A. (1967). Formation of gossypol in infected or chemically irritated tissues of *Gossypium* species. *Phytopathology*, **57:** 759-764.

Bell, A.A. (1969). Phytoalexin production and *Verticillium* wilt resistance in cotton. *Phytopathology*, **59:** 1119-1127.

Bhattacharyya, M.K. and Ward, E.W. (1988). Phenylalanine ammonia-lyase activity in soybean hypocotyls and leaves following infection with *Phytophthora megasperma* f. sp. *glycinea. Canadian Journal of Botany*, **66:** 18-23.

Bishop, C.D. (1980). Ultrastructure of vascular diseases. Ph.D. Thesis, University of Bath.

Bishop, C.D. and **Cooper, R.M.** (1983). An ultrastructural Study of vascular colonization in three vascular wilt diseases **I.** Colonization of susceptible cultivars. *Physiological Plant Pathology,* **23:** 323-343.

Bishop, C.D. and **Cooper, R.M.** (1984). Ultrastructure of vascular colonization by fungal wilt pathogens. 11. Invation of resistant cultivars. Physiological Plant of resistant cultivars. *Physiological Plant Pathology*, **24:** 277-289.

Blackhurst, F.M. and **Wood, R.K.S.** (1963). Resistance of tomato plants to *Verticillium albo-atrum. Transactions of the British Mycological Society,* **46:** 385-392.

Brettel, R.I.S. and **Ingram, D.S.** (1979). Tissue culture in production of novel disease resistant plants. *Biological Review*, **54:** 329-345.

Brinker, A.M. and **Seigler, D.S.** (1993). Time course of piceatannol accumulation in resistant and susceptible sugar cane stalks after inoculation with *Colletotricum falcatum. Physiological and Molecular Plant Pathology*, **42:** 169-176.

Bruegger, B. and **Keen N.T.** (1979). Specific elicitors of glyceollin accumulation in the *Pseudomonus glycinea* - soybean host-parasite system. *Physiological Plant Pathology,* **15:** 43-51.

Buddenhagen, I.W. (1990). Banana breeding and *Fusarium* wilt. In: Ploetz, R.C., ed. *Fusarium* Wilt of Banana. St. Paul: *American Phytopathological Society,* 107-114.

Bugbee, W.M. (1970). Vascular response of cotton to infection by *Fusarium oxysporum* f. sp. *vasinfectum. Phytopathology,* **60:** 121-123.

Burdett, A.N. (1970). The cause of bent neck in cut roses. *Journal of the American Society for Horticultural* Science, **95:** 427-431.

Cahill, D.M. and **McComb, J.A.** (1992). A comparison of changes in phynylalanine ammonia-lyase activity, lignin and phenolic synthesis in the roots of *Eucalyptus calophylla* (field resistant) and *E. marginata* (susceptible) when infected with *Phytophthora cinnamomi. Physiological and Molecular Plant Pathology,* **40:** 315-332.

Carder, J.H., Hignett, R.C. and **Swinborne, T.R.** (1987). The relationship between virulence of hop isolates of *Verticillium albo-atrum* and their *in vitro* secretion of cell wall degrading enzymes. *Physiological and Molecular Plant Pathology,* **31:** 441-452.

Carlson, P.S. (1973). Methionine sulfoximine-resistant mutants of tobacco. *Science,* **180:** 1366-1368.

Carpenter, J.B. (1971). Notes on Bayoud disease of date palms in Algeria. *Report Date Growers Institute,* **48:** 14-15.

Cho, Y.S., Wilconxson, R.D. and **Frosheiser, F.I.** (1973). Differences in anatomy, plant extract and movement of bacteria in plants of bacterial wilt resistant and susceptible varieties of alfalfa. *Phytopathology*, **63:** 760-765.

Colhoun, J. (1981). Vascular wilt disease of oil palms. In: Nelson, P.E., Toussoun, T.A., Cook, R.J., eds. *Fusarium* Disease, Biology and *Taxonomy.* University Park: Pennsylvania State University Press, 22-28.

Collinge, D.B., Milligan, D.E., Dow, J.M., Scofield, G. and **Daniel, M.J.** (1987). Gene expression in *Brassica campestris* showing a hypersensitive response to the incompatible pathogen *Xanthomonas compestris* pv *vitians. Plant Molecular Biology*, **8:** 405-411.

Cooper, R.M. (1974). Cell wall-degrading enzymes in vascular wilt diseases. Ph. D. Thesis, University of London.

*uusarium***Cooper, R.M.** (1977). Regulation of synthesis of cell-degrading enzymes of plant pathogens. In: *Cell Wall Biochemistry Related to Specificity in Host-Plant Pathogen Interactions.* (Eds. B. Solheim and J. Raa), Universitets-forlaget, Oslo, pp 163-211.

Cooper, R.M. (1981). Pathogen-induced changes in host ultrastracture. In: *Plant Disease Control: Resistance and Susceptibility.* Ed. by Staples, R.C. pp. 105-142. Wiley, J. and Sons Ltd.

Cooper, R.M. (1983). The mechanisms and significance of enzymic degradation of host cell walls by parasites. In: *Biochemical Plant Pathology*, ed. Callow, J.A., pp. 101-137. Wiley.

Cooper, R.M. (1984). The role of cell wall-degrading enzymes in infection and damage. In: Plant Diseases: *Infection, Damage and Loss* (eds. Jellis, J.G. and Wood, R.K.S.), Blackwell Scientific Publications, Oxford, pp. 13-27.

Cooper, R.M. and **Wood, R.K.S.** (1973). Induction of synthesis of extracellular cell wall-degrading enzymes in vascular wilt fungi. *Nature*, **246:** 309-311.

Cooper, R.M. and **Wood, R.K.S.** (1975). Regulation of synthesis of cell wall degrading enzymes by *Verticillium albo-atrum* and *Fusarium oxysporum* f. sp. *lycopersici. Physiological Plant Pathology*, **5:** 135-156.

Cooper, R.M., Rankin, B. and **Wood, R.K.S.** (1978). Cell wall-degrading enzymes. 11. properties and modes of action of polysaccharidases of *Verticillium albo-atrum* and *Fusarium oxysporum* f. sp. *lycopersici. Physiological Plant Pathology*, **13:** 101-134.

Cooper, R.M. and **Wood , R.K.S.** (1980). Cell wall-degrading enzymes of vascular wilt fungi 111. Possible involvement of endopectinlyase in *Verticillium* wilt of tomato. *Physiological Plant Pathology*, **16:** 285-300.

Cooper R.M., Flood, J. and **Mepsted R.** (1989). *Fusarium* wilt of oil palm: transmission, isolate variation and resistance. In: *Vascular wilt Diseases of Plants : Basic Studies and Control._* ed. E.C. Tjamos and C.H. Beckman. Springer-Verlag. pp. 247-258.

Correll, J.C., Puhalla, J.E. and **Schneider, R.W.** (1986). Vegetative compatibility groups among non-pathogenic root-colonizing strains of *Fusarium oxysporum. Canadian Journal of Botany*, **64:** 2358-2361.

Corsini, D.I. and **Pavek, J.J.** (1980). Phenylalanine ammonia lyase activity and fungi toxic metabolites produced by potatio cultivars in response to *Fusarium* tuber rot. *Physiological Plant Pathology,* **16:** 63-72.

Cruickshank, I.A.M. and **Perrin, D.R.** (1963 b). Studies on phytoalexins. VI. Pisatin: the effect of some factors on its formation in *Pisum sativum* L., and the significance of pisatin in disease resistance. *Australian Journal of Biological Sciences*, **16:** 111.

Dawson, R.M.C., Elliott, D.C., Elliott, W.H. and **Jones, K.M.** (1986). *Data for Biochemical Research,* 3rd edition. Oxford: Clarendon Press. pp. 482-485.

Deese, D.C. and **Stahmann, M.A.** (1962). Pectic enzymes in *Fusarium* infected susceptible and resistant tomato plants. *Phytopathology,* **52:** 255-260.

Daub, M.E. (1986). Tissue culture and selection of resistance to pathogens. *Annual Review of Phytopathology,* **24:** 159-186.

Deaton, W.R., Keyes, G.I. and **Collins, G.B.** (1982). Expressed resistance to black shank among tobacco callus cultures. *Theoretical Applied Genetics*, **63:** 65-70.

Diabete, S., Taquet, B., Renard, J.L., de Fraqueville, H. and **Reiser, P.** (1990). HPLC analysis of substances produced by oil palm and implicated in resistance to *Fusarium oxysporum* f. sp. *elaeidis;* prospects for selection. *Oléagineux,* **45:** *49-55.*

Dingle, J., Reid, W.W. and **Solomons, G.L.** (1953). The enzymic degradation of pectin and other polysaccharides. II Application of the 'cup plate' assay to the estimation of enzymes. *Journal of Science and Food Agriculture,* **4:** 149-154.

Dixon, R.A. (1986). The phytoalexin response: elicitation, signalling and control of host gene expression. *Biological Review,* **61:** 239-291.

Dixon, G.R. and **Pegg, G.R.** (1969). Hyphal lysis and tylose formation in tomato cultivars infected by *Verticillium albo-atrum. Transactions of the British Mycological Society,* **53 (1):** 109-118.

Djerbi, M. (1982). Bayoud disease in North Africa: history, distribution, diagnosis and control. *Date Palm Journal* **1 (2):** 153-197.

Dow, J.M., Scofield, G., Trafford, K., Turner, P.C. and **Daniels, M.J.** (1987). A gene cluster in *Xanthomonas campetris* pv *campetris* required for pathogenicity controls the excretion of polygalacturonate lyase and other enzymes. *Physiological and Molecular Plant Pathology,* **31:** 261-271.

Durbin, R.D. (1981). Applications. In: Toxins in Plant Disease (ed. R.D. Durbin) pp. 495-565. Academic Press, New York.

Durands, P.K. and **Cooper, R.M.** (1988). The role of pectinases vascular wilt diseases as determined by defined mutants of *Verticillium albo-atrum. Physiological and Molecular Plant Pathology,* **32:** 363-371.

Ebel, J., Schmidt, W.E. and **Loyal, R.** (1984). Phytoalexin synthesis in soybean cells: elicitor induction of phenylalanine ammonia-lyase and chalcone synthase mRNAs and correlation with phytoalexin accumulation. *Archives of Biochemistry and Biophysics,*
232: 240-248.

Elgersma, D.M. (1970). Length and diameter of xylem *vessels* as a factor in resistance of elms to *Ceratocystis ulmi. Netherlands Journal of Plant Pathology,* **76:** 179-182.

Elgersma, D.M. (1973). Tylose formation in elms after inoculation with *Ceratocystis ulmi*: a possible resistance mechanism. *Netherlands Journal of Plant Pathology,* **79:** 218-220.

Elgersma, D.M., Machardy, W.E. and **Beckman, C.H.** (1972). Growth and distribution of *Fusarium oxysporum* f. sp. *lycopersici* in near isogenic lines of tomato resistant and susceptible to wilt. *Phytopathology,* **62:** 1232-1237.

Elias, K.S., Schneider, R.W. and **Lear, M.M.** (1991). Analysis of vegetative compatibility groups in nonpathogenic populations of *Fusarium oxysporum* isolated from symptomless tomato roots. *Canadian Journal of Botany*, **69:** 2089-2094.

Fernandez, N., Patino, B. and **Vazquwz, C.** (1993). Pectin degrading enzymes secreted by six isolates of *Fusarium oxysporum. Mycological Research*, **97 (4):** 461-466.

Flood, J., Khan, F.z. and **Milton, J.M.** (1978). The role of phytoalexins in *Verticillium* wilt of lucerne. *Annals of Applied Biology*, **89:** 329-332.

Flood, J. and **Milton, J.M.** (1982). Studies on the incompatible/compatible interactins between lucerne leaves and *Verticillium albo-atrum, Physiological Plant Pathology,* **21:** 97-104.

Flood, J., Cooper, R.M. and **Lees, P.E.** (1989). An investigation of pathogenicity of four isolates of *Fusarium oxysporum* from South America, Africa and Malaysia to clonal oil palm. *Journal of Phytopathology*, **124:** 80-88.

Flood, J., Mepsted, R. and **Cooper, R.M.** (1990). Contamination of oil palm pollen and seed by *Fusarium* species. *Mycological Research*, **94:** 708-709.

Flood, J. Whitehead, D.S. and **Cooper, R.M.** (1992 a). Vegetative compatibility in *Fusarium oxysporum* f. sp. *elaeidis* and their relationship to isolate virulence and origin. *Physiological and Molecular Plant Pathology,* **41:** 201-215.

Flood, J. Mepsted, R, and **Cooper, R.M.** (1992 b). Potential spread of *Fusarium* wilt of oil palm on contaminated seed and pollen. *Proceedings of the Third International Conference on Plant Protection in the Tropics,* Kuala Lumpar, March 1990.
pp. 102-107.

Flood, J., Mepsted, R., Velez, A., Paul, T.C. and **Cooper, R.M.** (1993). Comparison of isolates of *Fusarium oxysporum* f. sp. *elaeidis* from Africa and South America. *Plant Pathology*, **42:** 168-171.

Flood, J., Mepsted, R, and **Cooper, R.M.** (1994). Population dynamics of *Fusarium* species on oil palm seeds following chemical and heat treatments. *Plant Pathology*, **42:** 177-182.

Franqueville (de), H. (1984). Vascular wilt of the oil palm: relationship between nursery and field resistance. *Oléagineux,* **39:** 513-518.

Franqueville (de), H. (1991). Former savannah or former forest: effect of pathogen isolate on the performance of oil palm families with respect to *Fusarium* wilt. *Oléagineux,* **46:** 179-186.

Franqueville (de), H. and **Greef (de), W.** (1987). Hereditary transmission of resistance to vascular wilt of the oil palm: Facts and hypotheses. *International Oil Palm Conference.* Kuala Lumpur.

Franqueville (de), H. and **Renard, J.L.** (1990). Improvement of palm vascular wilt tolerance. Results and development of the disease at the R. Michaux plantation. *Oléagineux,* **45:** 399-405.

Franqueville (de), H., Diabate, S., Renard, J.L. and **Jacquemard, J. Ch.** (1991). Assessment of oil palm clone performance with regard to vascular wilt. *British Society for Plant Pathology, Annual Conference,* 1991.

Fraselle, J.V. (1951). Experimental evidence of the pathogenicity of *Fusarium oxysporum* to the oil palm (*Elaeis guineensis). Nature,* **167:** 447.

Garas, N.A. and **Waiss, A.C. Jr.** (1986). Differential accumulation and distribution of antifungal sesquiterpenoids in cotton stems inoculated with *Verticillium dahliae. Phytopathology,* **75:** 1011-1017.

Garas, N.A., Wilhem, S. and **Sagen, J.E.** (1986). Relation of cultivar resistance to distribution of *Verticillium dahliae* in inoculated cotton plants and to growth of single conidia on excised stem segments. *Phytopathology,* **76:** 1005-1010.

Gerdemann, J.W. and **Finley, A.M. (1951).** The pathogenicity of race 1 and 2 of *Fusarium oxysporum* f. sp. *lycopersici. Phytopathology,* **41:** 238-244.

Goth, R.W. and **Webb, R.E.** (1981). Sources and genetics of resistance in vegetable crops. In: Mace, M.E., Bell, A.A. and Beckman, C.H., eds. *Fungal Wilt Diseases of Plants.* New York: Academic Press, 377-412.

Grzelinska, A. (1970). Peroxidase isoenzymes in Fusarium-infected tomato plants. *Phytopathologische Zeitschrift,* **69:** 211-222.

Guldentops, R.E. (1962). Contribution a l'etude de la tracheomycose du palmier a hile. *Parasitica,* **18:** 244-263.

Haberlach, G.T., Budde, A.D., Sequeira, L. and **Helgeson, J.P.** (1978). Modification of disease resistance of tobacco callus tissues by cytokinins. *Plant Physiology,* **62:** 522 - 525.

Hadwiger, L.A. and **Loschke, D.C.** (1981). Molecular communication in host-parasite interactions: hexosamine polymers (chitosan) as regulator compounds in race-specific and other interactions. *Phytopayhology,* **71:** 756-761.

Hahlbrock, K. and **Scheel, D.** (1989). Physiology and molecular biology of phenylpropanoid metabolism. *Annual Review of Plant Physiology and Plant Molecular Biology,* **40:** 347-369.

Hall, J.A. and **Wood, R.K.S.** (1973). The killing of plant cells by pectolytic enzymes. In: plant cells by pectolytic enzymes. In: Byrde, R.J.S. and Cutting, C.V. eds. *Fungal Pathogenicity and the Plant's Response.* London and New York: Academic Press, 19-38.

Hammerschlag, F. and **Mace, M.E.** (1975). Antifungal activity of extracts from *Fusarium* wilt susceptible and resistant tomato plants. *Phytopathology*, **65:** 93-94.

Hammerschmidt, R. and **Kuc, J.** (1982). Lignification as a mechanism for induced systemic resistance in cucumber. *Physiological Plant Pathology,* **20:** 61-71.

Hancock, J.G., Millar, R.L. and **Lorbeer, J.W.** (1964 a). Pectolytic and cellulolytic enzymes produced by *Botrytis allii, B. cinerea* and *B. squamosa in vitro* and *in vivo.* Phytopathology, **54:** 928-931.

Harborne, J.B. (1984). *Phytochemical methods, a Guide to Modern Techniques of Plant Analysis,* 2nd ed., Chapman and Hall, London, pp. 288.

Hargreaves, J.A., Mansfield, J.W., and **Rossall, S.** (1977). Changes in phytoalexin concentration in tissues of the broad bean plant *(Vicia faba L.)* following inoculation with species of *Botrytis. Physiological Plant Pathology,* **11:** 227-242.

Harrison, N.A. and **Beckman, C.H.** (1982). Time/space relationships of colonization and host response in wilt-resistant and wilt-susceptible cotton *(Gossypium)* cultivars inoculated with *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *vasinfectum. Plant Pathology,* **21:** 193-207.

Harrison, N.A. and **Beckman, C.H.** (1987). Growth inhibitors associated with *fusarium* wilt of tomato. *Physiological and Molecular Plant Pathology,* **30:** 401-420.

Hartley, C.W.S. (1988). *The Oil Palm.* Third edition. London: Longmans.

Heale, J.B. and **Cupta, D.P.** (1972). Mechanisms of vascular wilting induced by *Verticillium albo-atrum. Transactions of the British Mycological Society,* **58:** 19-28.

Helgeson, J.P., Kemp, J.D., Haberlach, G.T. and **Maxwell, D.P.** (1972). A tissue culture system for studying disease resistance: the Black Shank disease in tobacco callus cultures. *Phytopathology,* **62:** 1439-1443.

Helgeson, J.P. and **Haberlach, G.T.** (1980). Disease resistance studies with tissue cultures. In: Ingram, D.S. and Helgeson, J.P., eds. *Tissue culture methods for plant pathologist.* Oxford: Blackwell Scientific Publications, 179-184.

Hignett, R.C. Carder, J.H., Leach, J.E., Roberts, A.L., Welsh, M.P. and Swinburne, T.R. (1984). *Verticillium* wilt *(V. albo-atrum)* of hops: host-parasite relations. Report of *East Malling Research Station* for 1983, 105-106.

Hislop, F.C., Keon, J.P.R. and Fielding, A.H. (1979). Effects of pectinlyase from *Monilinia fructigena* on viability, ultrastructure and localization of acid phophatase of cultured apple cells. *Physiological Plant Pathology,* **14:** 371-381.

Ho, Y.W. and Taylor, G.S. 91985 a). Histopathology of oil palm seedlings infected by pathogenic *Fusarium oxysporum* isolates from Africa. *Phytopathology Zeitschrift,* **114:** 312-324.

Ho, Y.W., Varghese, G. and Taylor, G.S. (1985 b). Pathogenicity *Fusarium oxysporum* f. sp. *elaeidis* from Malaysia and *Fusarium oxysporum* f. sp. *elaeidis* from Africa to seedlings of oil palm *(Elaeis guineensis). Phytopathology Zeitschrift,* **114:** 193-202.

Ho, Y.W. and Varghese, G. (1986). Pathogenic potential of soil Fusaria from Malaysian oil palm habitatas. *Journey of Phytopathology,* **115:** 325-331.

Holliday, M.J. and Klarman, W.L. (1979). Expression of disease reaction type in soybean callus fro resistant and susceptible plants. *Phytopathology,* **69:** 576-578.

Holtz, G. and Knox-Davies, P.S. (1985 b). Pectic enzyme production by *Fusarium oxysporum* f. sp. *cepae:* induction by cell walls from different parts of onion bulbs at different growth stages. *Phytopathologische Zeitschrift,* **112:** 81-92.

Howell, C.R. (1976). Use of enzyme-deficient mutants of *Verticillium dahliae* to assess the importance of pectolytic enzymes in symptom expression of *Verticillium* wilt of cotton. *Physiological Plant Pathology,* **9:** 279-283.

Hutson, I.R.A. and **Smith, I.M**. (1980). Phytoalexins and tyloses in tomato cultivar infected with *Fusarium oxysporum* f. sp. *lycopersici* and *Verticillium albo-atrum. Physiological Plant Pathology*, **17:** 245 - 257.

Ingram, D.S. and Joachim, I. (1971). The growth of *Peronospora farinosa* f. sp. *betae* and sugar beet callus tissue in dual culture. *Journal of Generral Microbiology,* **69:** 211-230.

Isaac, S. (1992). *Fungal-Plant Interactions.* London: Chapman and Hall, 120-122.

Jones, D.H. (1984). Phenylalanine ammonia-lyase: Regulation of its induction and its role in plant developments. *Phytochemistry,* **23 (7):** 1349-1359.

Jones, L.H. (1983). The oil palm and its clonal propagation by tissue culture. *Biologist*, **30:** 181.

Jordan, C.M., Jordan, L.S. and Endo, R.M. (1988). Association of phenol-containing structures with *Apium graveolens* resistant to *Fusarium oxysporum* f. sp. *apii* race 2. *Canadian Journal of Botany,* **67:** 3153-3163.

Keen, N.T. and Legrand, M. (1980). Surface glycoproteins: evidence that they may funtion as the race specific phytoalexin elicitors of *Phytophthora megasperma* f. sp. *glycinea. Physiological Plant Pathology,* **17:** 175-192.

Keen, N.T. and Yoshikawa, M. (1938). B-1, 3- endoglucanase from soybean releases elicitor-active carbohydrates from fungus cell walls. *Plant Physiology,* **71:** 460-465.

Killian, C. and Maire, R. (1930). Beyoud, the disease of date palm. *Abstract Review of Applied Mycology,* **10:** 99-100.

Kovachich, W.G. Heusser, D. and Wimmer, H. (1948). A preliminary anatomical note on vascular wilt disease of oil palm *(Elaeis guineensis). Annals of Botany,* **12:** 327-330.

Krebs, K.G., Heusser, D. and Wimmer, H. (1969). Spray Reagents In: *Thin-Layer Chromatography, A Laboratory Handbook,* ed. Stahl, E. Springer-Verlag, New York, pp. 854-909.

Kroon, B.A.M., Scheffer, R.J. and Elgersma, D.M. (1991). Interactions between *Fusarium oxysporum* f. sp. *lycopersici* and callus of susceptible and resistant tomato lines: Fungal growth and phytoalexin accumulation. *Journal of Phytopathology,* **132:** 57-64.

Kroon, B.A.M. and Elgesma, D.M. (1993). Interactions between races 2 of *Fusarium oxysporum* f. sp. *lycopersici* and nearisogenic resistant and susceptible lines of intact plants or callus of tomato. *Journal of Phytopathology,* **137:** 1-9.

Kullaya, A. (1991). Oil palm production in Tanzania. In: Anon, (1991). The Role of Research Networks for the Development of Oil Palm in Africa. AFOPDA/BUROTROP seminar. Abidjan. June (1991). Paris. BUROTROP.

LANDE (Van de), H.L. (1984). Vascular wilt disease of oil palm (*Elaeis guineensis)* in Para, Brazil. *Oil Palm News,* **28:** 6-10.

Latunde-Dada, A.O. and Lucas, J.A. (1985). Involvement of the phytoalexin medicarpin in the differential response of callus lines of lucerne (*Medicago sativa)* to infection by *Verticillium albo-atrum. Physiological Plant Pathology,* **26:** 31-42.

Link, K.P., Dickson, A.D. and Walker, J.C. (1929). Further observation on the occurrence of protocatechuic acid in pigmented anion scales and its relation to disease resistance in onions. *Journal of Biological Chemistry,* **84:** 719-729.

Lobenstein, G. and Linsey, N. (1961). Peroxidase activity in virus-infected sweet potatoes. *Phytopathology,* **51:** 533-537.

Locke, T., Colhoun, J. (1977). A process of infection of oil palm seedlings by *Fusarium oxysporum* f. sp. *elaeidis. Phytopayhologische Zeitschrift,* **88:** 18-28.

Loffler, H.J.M. and Rumine, P. (1991). Virulence and vegetative compatibility of Dutch and Italian isolates *Fusarium oxysporum* f. sp. *lilii. Journal of Phytopathology,* **132:** 12-2.

Louvet, J. (1988). *Fusarium oxysporum.* In: Smith, I.M., Dunez, J., Phillip, D.H., Lelliot, R.A. and Archer, S.A., eds. *The European Handbook of Plant Disease.* London: Blackwell Scientific Publications, 289-292.

MacDonald, W.L. and McNabb, H.S. (1970). Fine structural observations of the growth of *Ceratocystis ulmi* in elm xylem tissue. *Bioscience,* **20:** 1060-1061.

McNabb, H.S. JR., Heybrook, H.M. and MacDonald, W.L. (1970). Anatomical factors in resistance to elm disease. *Netherlands Journal of Plant Pathology,* **76:** 196-204.

Mace, M.E. (1978). Contributions of tyloses and terpenoid aldehyde phytoalexins to *Verticillium* wilt resistance in cotton. *Physiological Plant Pathology, 12:* 1-11.

Mace, M.E., Veech, J.A. and Hammerschlag, F. (1971). *Fusarium* wilt of susceptible and resistant tomato isolines: spore transport. *Phytopathology,* **61:** 627-630.

Malencon, G. (1941). L'infection florale du dattier par la *Fusarium albedinis* (Kill. et Maire) Mlcn. C.R. *Academic Science, Paris,* **223:** 923-925.

Malencon, G. (1950). La diffusion et l'epidemiologie de la maladie fusrienne du palmier-dattier en Afrique du Nord. *Rev. Mycol. Suppl. Colon.,* **14 (1):** 45-60.

Mansfield, J.W. (1983). Antimicrobial compounds. In: *Biochemical Plant Pathology,* ed. Callow, J.A.; John Wiley & Sons Ltd., Chichester, pp. 237-265.

Mansfield, J.W. and Bailey, J.A. (1982). Phytoalexins: current problems and future prospects. In: *Phytoalexins,* eds. Bailey, J.A. and Mansfield, J.W.; Blackie & Son Ltd., Glasgow, pp. 319-223.

Maraite, H. (1973). Changes in polyphenoloxidases and peroxidases in muskmelon *(Cucumis melo)* infected by *Fusarium oxysporum* f. sp. *melonis. Physiological Plant Pathology,* **3:** 29-49.

Marley, P. and Hillocks, R. (1993). The role of phytoalexins in resistance to *Fusarium* wilt in pigeon pea *(Cajanus cajan). Plant Pathology,* **42:** 212-218.

Matta, A. (1971). Microbial penetration and immunization of uncongenial host plants. *Annual Review of Phytopathology,* **9:** 387-410.

Matta, A. and Diamond, A.E. (1963). Symptoms of *Fusarium* wilt in relation to quantity of fungus and enzyme activity in tomato stems. *Phytopathology,* **53:** 574-578.

Mauch, F., Mauch-Mani, B. and Boller, T. (1988). Antifungal hydrolases in pea tissue 11. Inhibition of fungal growth by combinations of chitinase and B, 1-3-glucanase. *Plant Physiology,* 936-942.

McNabb, H.S., Heybrock, H.M. and MacDonald, W.L. (1970). Anatomical factors in resistance to Dutch elm disease. Netherlands Journal of Plant Pathology, **76:** 196-204.

Mead, R. and Curnow, R.N. (1983). *Statistical Methods in Agriculture and Experimental Biology.* London: Chapman and Hall.

Mepsted, R.C. (1992). Studies on *Fusarium* wilt of oil palm. Ph. D. Thesis, University of Bath.

Mepsted, R.C., Flood, J., Paul, T.C. and Cooper, R.M. (1994). Virulence and aggressiveness in *Fusarium oxysporum* f. sp. *elaeidis;* implications for screening for disease; resistance. *Oléagineux,* **49:** 209-211

Mepsted, R.C, Flood, J., Paul T.C., Airede, C. and Cooper, R.M. (1995). A model system for rapid selection of resistance and for investigation of resistance mechanisms in Fusarium wilt of oil palm. *Plant Pathology* , **44:** 749 - 755.

Meunier, J., Renard, J.L. and Quillec, G. (1979). Heredity of resistance to *Fusarium* wilt in the oil palm *Elaeis guineensis* Jacq. *Oléagineux,* **34:** 555-561.

Miller, S.A., Davidse, L.C. and Maxwell, D.P. (1984). Expression of genetic susceptibility, host resistance, and non-host resistance in *alfalfa* callus tissue inoculated with *phytophthora megasperma. Phytopathology,* **74:** 345-348.

Mollenhauer, H.H. (1976). Xylem morphology of pierce's disease infected grape vines with different levels of tolerance. *Physiological Plant Pathology,* **9:** 95 - 100.

Mouyna, I., Renard, J.R. and Brygoo, **Y.** (1994). Characterization and DNA polymorphism in *Fusarium oxysporum* f.sp. *elaeidis* isolated from oil palm. In: *Rapport final* defence reaction mechanism in oil palm with respect to *Fusarium oxysporum* f. sp. *elaeidis*. Cytological studies and biochemical analyses. Application to the srengthening of selection criteria. Ed. Renard, J.L. pp. 1 - 11. CIRAD-CP Paris.

Moreau, C., and Moreau, M. (1960). Inhibition de la croissance du *Fusarium oxysporum* Schl. par divers fongicides organiques. *Review of Mycology,* Paris, **25:** 307-310.

Moreau, M., Catesson, A.M., Peresse, M. and Czaninski, Y. (1978). Comparative dynamics of xylem reactions in cornation toward vascular parasite. *Phytopathologische Zeitschrift,* **91:** 289-306.

Mozzetti, C., Ferraris, L., Tamietti, G. and Matta, A. (1995). Variation in enzyme activities in leaves and cell suspensions as markers of incompatibility in different *Phytophthora*-pepper interactions *Physiological* and *Molecular Plant Pathology,* **46:** 95-107.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiological Plantarum,* **15:** 473-497.

Newcombe, A.G. and Robb, J. (1988). The function and relative importance of the cascular coating response in highly resistant, moderately resistant and susceptible alfalfa infected by *Verticillium albo-atrum. Physiological and Molecular Plant Pathology,* **33:** 47-58.

Newcombe, A.G., Papadopoulos, Y.A., Robb, J. and Christie, B.R. (1989). The colonization ratio: a measure of pathogen invasiveness and host resistance in *Verticillium* wilt of alfalfa. *Canadian Journal of Botany,* **67:** 365-370.

Obuekwe, C.O. and Osagie, I.J. (1989). Morphological changes in infected wilt resistant and wilt susceptible oil palm progenies and hydrolytic enzyme activities associated with the *Fusarium oxysporum* f. sp. *elaeidis* pathogens. *Oléagineux,* **44:** 392-401.

O'Garro, L.W. and Clarkson, J.M. (1988). Pathogenicity of race 1 and race 2 isolates of *Verticillium dahliae* from different geographical origins. *Journal of Phytopathology,* **123:** 297-303.

Oritsejafor, J.J. (1989). Status of the oil palm vascular wilt disease in Nigeria. *Internation Conference on oil palm and Palm Products.* Benin City: NIFOR.

Osagie, I.J. and Obuekwe, C.O. (1991). Extracellular hydrolytic enzyme production by pathogenic strains of *Fusarium oxysporum* f. sp. *elaeidis. Mycological Research,* **95:** 116-122.

Osbourn, A., Bowyer, P., Bryan, G., Lunness, P., Clarke, B. and Daniels, M. (1994). Detoxification of plant saponins by fungi. In: *Advances in Molecular Genetics of Plant-Microbe Interactins,* eds. Daniels, M.J. *et al.* Kluwer Academic Publishers, Netherlands, **3:** 215-221.

Papavizas, G.C. (1967). Evaluation of various media and antimicrobial agents for isolation of Fusaria from soil. *Phytopathology,* **57:** 848-852.

Park, D. (1958). The saprophytic status of *Fusarium oxysporum* Schl. causing vascular wilt of oil palm. *Annals of Botany,* **22:** 19-35.

Parker, R.E. (1979). *Introductory Statistics in Biology,* 2nd edition. London: Edward Arnold.

Paxton, J.D. (1981). Phytoalexins a working redefinition. *Phytopathologische Zeitsschrift,* **101:** 106-109.

Pegg, G.F. (1974). *Verticillium* diseases. *Review of Plant Pathology,* **53:** 157-182.

Pereau-Leroy, P. (1958). Le palmier-dattier au Maroc, *IFAC, paris, et Minist. d'Agric.,* Rabat, Moroc. pp. 142.

Prendergast, A.G. (1957). Observations on the epidemiology of vascular wilt disease of the oil palm *(Elaeis guineensis,* Jacq.). *Journal of Research,* **2:** 148-175.

Prendergast, A.G. (1963). A method of testing oil palm progenies at the nursery stage for resistance to vascular wilt disease caused by *Fusarium oxysporum* Schl. *Journal Palm Research,* **4:** 156 - 175.

Presley, J.T. and Taylor, E.E. (1969). Ontogeny of vessels influence the development of *Verticillium* influence the development of *Verticillium* in young cotton plants. *Phytopathology,* **59:** 253-254.

Presley, J.T., Cairns, H.R., Taylor, E.E. and Schnathorst, W.C. (1966). Movement of conidia in *Verticillium albo-atrum* in cotton plants. *Phytopathology,* **56:** 375.

Puhalla, J.E. and Howell, C.F. (1975). Significance of endopolygalacturonase activity to symptom expression of Verticillium wilt in cotton, assessed by the use of mutants of *Verticillium dahliae* Kleb. *Physiological Plant Pathology,* **7:** 147-152.

Purseglove, J.W. (1985). Tropical Crops: Monocotyledons. *Elaeis guineensis* Jacq. *Journal of Experimental Botany,* **12:** 129.

Reed, S.M. and Rufty, R.C. (1985). Reaction of calli of blue mold resistant and susceptible *Nicotiana* genotypes to infection by *Peronospora tabacina. Tobacco Science,* **29:** 53 - 56.

Renard, J.L. (1970). La fusariose du palmier a huile. Role des blessures des racines dans le processus d'infection. *Oléagineux,* **25:** 581-586.

Renard, J.L. (1973). Transport et distribution du bemomyl dans les palmier a hile au stade pé[omiere. *Oléagineux* **28:** 557-562.

Renard, J.L. (1976). Disease in Africa and South America. In Corley, R.H.V., Hardon, J.J. and Wood, B.J. eds. *Developments in Crop Science (1), Oil Palm Research.* Amsterdam: Elsevier Scientific Publishing Company, 447-466.

Renard, J.L., Gascon, J.P. and Bachy, A. (1972). Research on vascular wilt disease of the oil palm. *Oléagineux,* **27:** 581-591.

Renard, J.L. and Franqueville(de), H. (1989). Oil palm vascular wilt. *Oléagineux,* **44:** 342-347.

Renard, J.L., Noiret, J.M. and Meunier, J. (1980). Sources and ranges of resistance to *Fusarium* wilt in the oil palms *Elaeis guineensis* and *Elaeis melanococca. Oléagineux,* **35:** 387-393.

Renard, J.L. and Quillec, G. (1983). *Fusarium* Aand rplanting. Elements to be considered when replanting oil palm in a *Fusarium* zone in West Africa. *Oléagineaux,* **38:** 421-427.

Retig, N. (1974). Changes in peroxidase and polyphenoloxidase associated with natural and induced resistance of tomato to *Fusarium* wilt. *Physiological Plant Pathology,* **4:** 145-150.

Resende, M.L.V. (1994). Verticillium wilt of cocoa, studies on pathogenicity and resistance. Ph. D. Thesis, University of Bath.

Robb, J., Brisson, J.D., Busch, L. and Lu, B.C. (1979 a). Ultra-structure of wilt syndrome caused by *Verticillium dahliae V1.* Correlated light and transmission electron microscope identification of vessel coating and tyloses. *Canadian Journal of Botany,* **57:** 822-834.

Robb, J., Powel, D. and Street, P.F.S. (1987). The chronological development of the coating response in *Verticillium*-infected tomato petioles. *Physiological and Molecular Plant Pathology,* **31:** 217-226.

Sanchez Potez, A. (1966). Enfermedades del Algodonero del cocofero y de la palma Africana en Colombia. In: *Review of Applied Mycology,* **46:** 630 (Abstract).

Scheffer, R.P. and **Walker, J.C.** (1953). The physiology of Fusarium wilt of tomato *Phytopathology,* **43:** 116-125.

Schneider, R.W. (1984). Effect of nonpathogenic strains of *Fusarium oxysporum* f. sp. *apii* and a novel use of the Lineweave-Burk double reciprocal plot technique. *Phytopathology,* **74:** 646-653.

Schlumbaum, A., Mauch, F., Vogeli, U. and **Boller, T.** (1986). Plant chitinases are potent inhibitors of fungal growth. *Nature,* **324:** 365 - 367.

Seigel, S. (1956). *Nonparametric Statistics for the Behavioral Sciences.* New York: McGraw-Hill Book Company.

Shi, J., Mueller, W.C. and Beckman, C.H. (1992). Vessel occlusion and secretory activities of vessel contact cells in resistant or susceptible cotton plants

infected with *Fusarium oxysporum* f. sp. *vasinfectum. Physiological and Molecular Plant Pathology,* **40:** 133-147.

Shi, J., Mueller, W.C. and Beckman, CH.H. (1993). The inhibition of fungal growth in resistant cotton plants infected by *Fusarium oxysporum* f. sp. *vasinfectum. Journal of Phytopathology,* **139:** 253-260.

Sinha, A.K. and Wood, R.K.S. (1967). An analysis of responses of resistant and of susceptible tomato plants to *Verticillium* infection. *Annals of Applied Biology,* **59:** 143-154.

Siha, A.K. and Wood, R.K.S. (1968). Studies on the nature of resistance in tomato plants to *Verticillium albo-atrum. Annals of Applied Biology,* **62:** 319-327.

Sokal, R.R. and Rohlf, F.J. (1981). *Biometry. The Principles and Practices of Statistics in Biological Research,* 2nd edition. London: Freeman and Company.

Sperry, J.S. (1986). Relationship of xylem embolism to xylem pressure potential, stomatal closure, and shoot morphology in the palm *Rhapis excelsa. Plant Physiology,* **80:** 110-116.

Stephen, G.J. and Wood, R.K.S. (1974). Release of ensymes from cell walls by an endopectate-transeliminase. *Nature,* **251:** 358.

Street, P.F.S., Robb, J. and Ellis, B.E. (1986). Secretion of vascular coating components by xylem parenchyma cells of tomato infected with *Verticillium albo-atrum. Protoplasma,* **132:** 1-11.

Stromberg, E.L. and Corden, M.E. (1977). Fungitoxicity of xylem extracts from tomato plants resistant or susceptible to *Fusarium* wilt. *Phytopathology,* **67:** 693-697.

Talboy, P.W. (1958 b). Association of tylosis and hyperplasia of the xylem with vascular invasion of the hop by *Verticillium albo-atrum. Transactions of British Mycological Society,* **41:** 249-260.

Talboys, P.W. (1972). Resistance to vascular wilt fungi. *Proceedings of the Royal Society, Series B,* **181:** 319-332.

Talboys, P.W. and Busch, L.V. (1970). Pectic enzymes produced by *Verticillium* species. *Transactions of the British Mycological Society,* **55:** 367-381.

Taquet, B., Ravise, A., Renard, J.L. and Kunesch, G. (1985). Modulation des reaction de defence du palmier a huile *(Elaeis guineensis)* contre le *Fusarium oxysporum* f. sp. *elaeidis. Phytopathologische Zeitscrift,* **112:** 298-314.

Teakle, D.S., Appleton, J., Steindl, D.R.L. (1978). An anatomical basis for resistance of sugar-cane to stunting disease. *Physiological Plant Pathology,*

12: 83-91.

Tessier, B.J., Mueller, W.C. and Morgham, A.T. (1990). Histopathology and ultrastructure of vascular responses in peas resistant or susceptible to *Fusarium oxysporum* f. sp. *pisi. Phytopathology,* **80:** 756-764.

Thurston, H.D. (1984). Tropical Plant Disease. *The American Phytopathological Society.*

Tjamos, E.C. and Smith, I.M. (1974). The role of phytoalexins in the resistance of tomato to *Verticillium* wilt. *Physiological Plant Pathology,* **4:** 249-259.

Tjamos, E.C. and Smith, I.M. (1975). The expression of resistance to *Verticillium albo-atrum* in monogenically resistant tomato varieties. *Physiological Plant Pathology,* **6:** 215-225.

Tomlinson, P.B. (1990). *The Structural Biology of Palms.* Oxford: Clarendon Press.

Tussoun, T.A. (1975). *Fusarium* suppressive soils. In: Biology and Control of Soil-Borne Plant Pathogens. Ed. Bruehl, G.W. *American Phytopathological Society,* pp. 145-151.

Trique, B. (1971). Pectinases et acide fusarique du *fusarium oxysporum* f. sp. *elaeidis,* leur role dans la fusariose du palmier a huile. *Oléagineux, 26: 163-168.*

Turner, P. (1981). *Oil Palm Diseases and Disorders.* Oxford: Oxford University Press.

Turner, S.L. (1992). *Studies of Fusarium Wilt of Oil Palm with Reference to Seed Decontamination and Soil Epidemiology.* Report for Honours Degree in Applied Biology. University of Bath.

Vandermolen, G.E. (1978). Electron microscopy of vascular obstrations in citrus roots affected with young tree decline. *Physiological Plant Pathology,* **13:** 271-274.

Vandermolen, G.E., Beckman, C.H. and Rodehorst, E. (1977). Vascular gelation: a general response phenomenon following infection. *Physiological Plant Pathology,* **11:** 95-100.

Vandermolen, G.E., Labavitch, J.M. and De Vay, J.E. (1982). Carbohydrate composition and structure of vascular gels from wilt-diseased banana roots. *Plant Physiology,* **69** (suppl.): 143.

Vandermolen, G.E., Labavitch, J.M., Strand, L.L and De Vay, J.E. (1983). Pathogen-induced vascular gels: ethylene as a host intermediate. *Physiological Plant Pathology,* **59:** 573-580.

Van den Bulk, R.W. (1991). Application of cell and tissue culture an *in vitro* selection for disease resistance breeding a review. *Euphytica,* **56:** 269-285.

Van de Lande, H.L. (1983). Vascular wilt disease of oil palm *(Elaeis guineensis* Jacq) in Para, Brazil. *Oil Palm News,* **27:** 6-10.

Vernenghi, A., Taquet, B., Renard, J.L. and Ravise, A. (1987). Detection in the oil palm of toxic polyenic fatty acids oxygenated drivatives for *Fusarium oxysporum* f. sp. *elaeidis;* variation in their accumulation according to crosses and methods of treatment. *Oléagineux,* **42 (1):** 1-10.

Wardlaw, C.W. (1946 b). *Fusarium oxysporum* on the oil palm. *Nature,* **158:** 712.

Wardlaw, C.W. (1946 c). *Observations on Diseases of the Oil Palm in the Belgian Congo.* Unilever Internal Report.

Wardlaw, C.W. (1948). Vascular wilt disease in Nigeria. *Nature,* **162:** 850-851.

Wardlaw, C.W. (1950). Vascular wilt disease of the oil palm caused by *Fusarium oxysporum* Schl. *Tropical Agriculture,* **27:** 42-47.

Wargo, P.M. (1975). Lysis of cell wall of *Armillaria mellea* by enzymes from forest trees. *Physiological Plant Pathology,* **5:** 99-105.

Waterson, J.M. (1953). Observations on the influence of some ecological factors on theincidence of oil palm diseases in Nigeia. *Journal of the West African Institute For Oil Palm Research,* **1:** 24-59.

Young, D.H. and Pegg, G.F. (1982). The action of tomato and *Verticillium albo-atrum* glycosidases on the hyphal wall of *Verticillium albo-atrum. Physiological Plant Pathology,* **21:** 411-423.

Yoshikawa, M., Masago, H., Onoe, T. and **Matsuda, K.** (1987). Mode of biochemical action of phytoalexins. In: *Molecular Determinants of Plant Disease.* Eds. Nishumura, S., Vance, C.P. and Doke, N., Springer-Verlag, Japan Scientific Societies Press, Tokyo, pp. 253 - 269.

Yu, K., Christie, B.R. and Pauls, K.P. (1993). A comparison of screening technique for resistance to *Verticillium* wilt in alfalfa *(Medicago sativa). Journal of Phytopathology,* **139:** 26-32.

Zarroogian, G.E. and Beckman, C.H. (1968). A comparison of cell wall composition in banana plants resistant or susceptible to *Fusarium oxysporum* f. sp. *cubense. Phytopathology,* **58:** 733-735.

Zhang, J., Mace, M.E., Stipanovic, R.D. and Bell, A.A. (1993). Production and fungitoxicity of the terpenoid phytoalexins in cotton inoculated with *Fusarium oxysporum* f. sp. *Vasinfectum. Journal of Phytopathology,* **139:** 247-252.

Zimmermann, M.H. and Sperry, J.S. (1983). Anatomy of the palm *Rhapis excelsa.* IX xylem structue of the leaf insertion *Journal of the Arnold Arboretum,* **64:** 599-609.

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I will always remember my family here in U. K. (Mariam, Michael, James, Roda and Isaac) for bearing witness to my long and hard struggle and also my relatives in my home town, Yambio, (Southern Sudan) who I have not been able to communicate with during this study.

Finally, I wish to acknowledge the financial support of the E. E. C. during the period of this research.

APPENDICES

Appendix 1

Preparation of media

All media were sterilized at 121 ^{0}C for 15 minutes.

1. **Sucrose (*Fusarium*) Salts Medium (FSM)** (Cooper and Wood, 1975).

	g l^{-1}
$NaNO_3$	2
KH_2PO_4	1
$MgSO_4.\ 7H_2O$	0.5
Sucrose	15
Trace element stock solution	10 ml l^{-1}

Trace element stock solution :-

$FeSO_4.\ 7H_{2O}$	20
$ZNSO_4.\ 2H_2O$	100
$NaMoO_4.\ 2H_2O$	2
$CUSO_4.\ 5H2O$	2
$MnCl_4.\ H2O$	2

The medium was made up to 1 Litre in distilled water and adjusted to pH 6.5 before sterilization.

2. Papavizas Medium (Fusarium Selective Medium) (Papavizas, 1967).

	$g\ l^{-1}$
KH_2PO_4	1
Peptone	5
$MgSO_4.\ 7H_2O$	0.5
Botrilex	5
(20% a. i. Pentachloranitrobenzene)	
Agar	12

The medium was made up to 1 Litre in distilled water. The following antimicrobial agents were added to the medium as it cooled after sterilization:- 0.05g Chloramphenicol, 0.3g Penicillin, 0.134g Streptomycin Sulphate.

3. Potato Dextrose Agar (PDA).

39g of PDA powder (London Analytical & Bacteriological Media Ltd., London) was added to 1 litre of distilled water.

4. MSSH - Growth Medium

MS (Marashige & Skooge, Sigma)	4.4g
Sucrose (Amersham)	20g
2,4,D (Sigma)	0.5mg
Kinetin (Sigma)	1.0g
Bacteriological Agar No. 1. (Oxoid)	15g
Distilled, Deionised Water	1000ml

pH 5.8 before autoclaving

Appendix 2

Mass production of inoculum of *Fusarium oxysporum*

A 3 litre flask containing 1.3 litres of sterile sucrose salts media (appendix 1) was inoculated with 140 ml of a spore suspention of 2.5 x 10^7 spores/ml. The flask was aerated by use of a pump blowing air through a sterile filter to an aquarium air stone in the media. After 3 days incubation at 27 ^{0}C, the flasks yielded 1.3 litres of spore suspention of 2 x 10^8 spores/ml. This would be enough to inoculate almost 800 plants with 10 ml of 3.3 x 10^7 spores/ml.

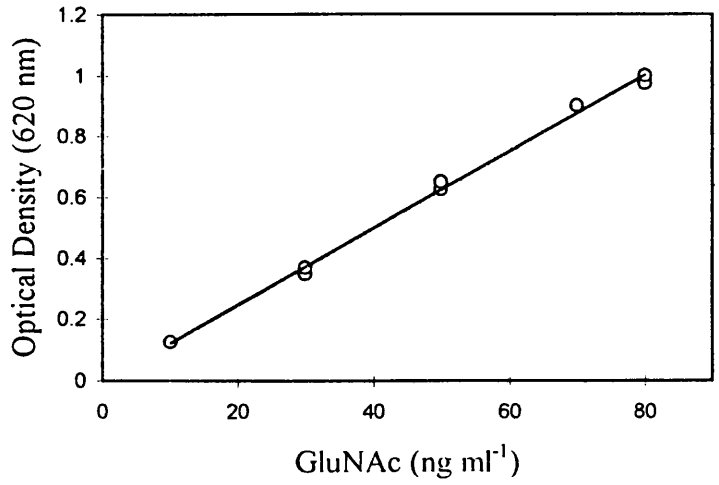
Appendix 3

Isolates of *F.o.e.* were imported and retained under licence (PHF 343/134 (107)) issued by the Minstry of Agriculture Fisheries and Food.

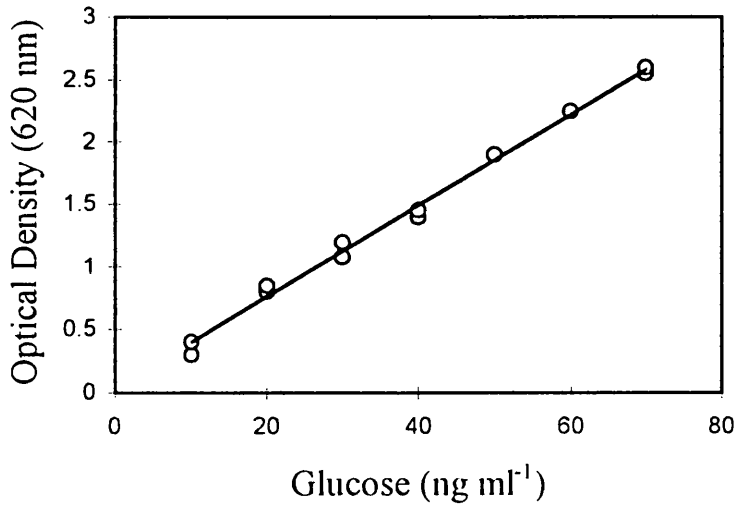
Cladosporium herbarum was supplied by Prof. J. Mansfield, Wye college University of London.

Appendix 4

Nacetylglucosamine Standard Curve



Glucose Standard Curve



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A model system for rapid selection for resistance and investigation of resistance mechanisms in *Fusarium* wilt of oil palm

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Fusarium wilt (*Fusarium oxysporum* f.sp *elaeidis*) is the most serious disease of oil palm in Africa. Control measures are dependent upon breeding for resistance, but the lack of a rapid screen for resistance and lack of understanding of the underlying resistance mechanisms has constrained breeding programmes. A novel petiole infusion technique is reported that exploits the ability of the majority of petiole cells to express disease resistance or susceptibility. A clear correlation was found between disease resistance or susceptibility of six clones and external symptoms and fungal colonization in petioles. Antifungal compounds accumulated in resistant but not in susceptible clones in response to inoculation; there was also some evidence of preformed antifungal compounds in resistant clones. Further investigation of resistance mechanisms can be undertaken using this model system. The use of this novel technique, both as a potential rapid replacement for current nursery selection methods (time reduced from 8 months to 8 days) and as an assessment of resistance in individual field palms, is discussed.*

INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *elaeidis* is the most important disease of oil palm (*Elaeis guineensis*) in Africa. The disease has been reported from many West African countries, including Zaire (Wardlaw, 1946), Nigeria (Wardlaw, 1948), Cameroon (Anon., 1960) and the Ivory Coast (Renard *et al.*, 1972), and widespread losses have occurred (Aderungboye, 1981). The disease has also been reported from Denpasa Estate, Brazil in 1983 (Van de Lande, 1984) and from Ecuador in 1986 (Renard & de Franqueville, 1989), and the pathogenicity of a Brazilian isolate to clonal oil palm has been established (Flood *et al.*, 1989). The Brazilian isolate is highly aggressive and can cause disease in oil palm lines selected for resistance in African breeding programmes; this has serious implications for the selection of breeding material for South America (Flood *et al.*, 1993).

Control measures are dependent upon breeding for resistance, but breeding programmes are constrained for a number of reasons. The inheritance of wilt resistance is still not fully understood, partly due to the lack of a rapid test for resistance both for young palms in the nursery (symptoms take 4–8 months to develop) and for individual palms in the field; the latter are used as sources of potentially resistant seed or as ortets for resistant clones. Furthermore, there is a lack of understanding of the underlying resistance mechanisms, knowledge of which could lead to more logical and facile screening methods. However, resistance mechanisms are difficult to study in this host-pathogen interaction because of the chronic, asynchronous nature of the disease and because the morphology of young palms offers few tissues to test as 'model systems'. The swollen stem base (pseudobulb) has a highly complex vascular architecture and problems of experimental replication arise with regard to the roots; removal of several roots from each plant can lead to plant death. We here report a technique which exploits the ability of cells in the petioles of oil palm to express disease resistance or susceptibility and which will act as a model system for rapid detection of disease-resistant lines and for studying resistance mechanisms in this disease.

*Since submission of the paper this technique has been used on field palms at Dabou, Côte D'Ivoire, where it successfully distinguished between resistant and susceptible clonal palms.

MATERIALS AND METHODS

Plant material

Plantlets of six oil palm clones were supplied by Unifield T C Ltd, Bedford, UK. The plantlets were hardened off in propagators by gradually reducing the humidity over a period of 2 months, after which they were individually potted into black polyethylene bags (80 mm × 190 mm) containing 1·2 l of compost (Levingtons F2, Levingtons M2, Perlite; ratio 1 : 1 : 1, Sinclair Horticulture Ltd, Lincoln, UK). The disease resistance of each clone was determined 6 months after soil inoculation with microconidia, by assessing the frequency of infection as reflected by vascular discoloration in the pseudobulb (Renard *et al.*, 1972; Flood *et al.*, 1989). All clones were assessed relative to a resistant standard R1.

Fungal isolates

F3, a representative pathogenic isolate of *F. oxysporum* f.sp. *elaeidis* from Binga, Zaire was used in the investigations. Cultures were stored in soil and maintained as described previously (Flood *et al.*, 1989).

Preparation of inoculum

For inoculation, isolate F3 was removed from soil culture, grown on PDA for 2 days, and then five mycelial plugs (8 mm in diameter) were added to 100 ml of sucrose-salts medium (Cooper & Wood, 1975). The cultures were maintained for 5 days at 100 rpm and 28°C and then filtered and centrifuged. Spores were resuspended in sterile distilled water and adjusted to 10^6/ml unless otherwise stated.

Inoculation methods

Several methods of infiltrating petioles were initially attempted, including transpiration under natural conditions and infusion by syringe, but these methods were abandoned in favour of infusion under reduced pressure. Hence, petiole segments (2·5 cm long) were cut with sterile razor blades from the youngest fully opened leaves of 1 to 2-year-old clonal palms. The petioles were infused with 0·5 ml of spore suspension under reduced pressure (1500 Pa). The first 1 mm from each inoculated end was then removed in order to reduce contamination. Control petioles were either infused with 0·5 ml of sterile distilled water (wet control) or incubated dry (dry control).

In most cases, petioles were incubated for 8 days in moist chambers at 28°C, after which external disease symptoms, internal fungal colonization and accumulation of antifungal compound(s) were assessed, but in some later experiments, petioles were incubated for 1–8 days in order to allow the assessment of antifungal accumulation with time.

Assessment of external symptoms

Following incubation, the petioles were scored for external symptoms using a scale of 0–3 where 0 = totally green, 0·5 = slight browning at one cut end, 1·0 = slight browning at both cut ends, 2·0 = browning more than 5 mm from cut end, and 3·0 = section totally brown.

Assessment of colonization of petioles

Fungal colonization was assessed by microscopic examination of replicate sections taken from the mid point of each petiole. In each section, the total number of hyphae observed in five randomly selected areas of $4 \times 10^3/\mu m^2$ were counted.

Extraction of antifungal compounds from petiole xylem fluids

To remove antifungal compounds from petiole xylem fluids, a glass syringe fitted with solvent-resistant 'Viton' tubing (external diameter 4·9 mm, Alltech Associated Applied Science Ltd, Carnforth, UK) was used to flush 1 ml of diethyl ether through the petiole segments; 50 μl of these xylem extracts were collected, placed on sterile cavity slides and evaporated to dryness prior to bioassay.

Extraction of antifungal compounds from petiole tissue

Petioles (4–5 g fresh weight) were comminuted with 2–3 ml of acid-washed sand plus 8–10 ml of diethyl ether. The extracts were evaporated to dryness and the residues dissolved in 0·25–0·5 ml of diethyl ether. Aliquots of 50 μl of these tissue extracts were placed on sterile cavity slides and evaporated to dryness prior to bioassay.

Determination of antifungal activity by spore bioassay

The antifungal activity of the extracts was

Table 1. External symptoms and fungal growth in petiole sections (S1) infused with different concentrations of *Fusarium oxysporum* f.sp.*elaeidis*, 8 days after treatment[a]

Inoculum (conidia/ml)	Symptoms (browning score)	Fungal growth in petiole[b]
10^6	2·1b[c]	92·6a
10^4	2·0b	93·4a
10^2	1·8b	90·0a
Control	0·2a	1·3b

[a]Values represent the mean of seven replicate plants; one petiole per plant and observations of three sections per petiole for fungal growth.
[b]Total number of hyphae observed in five randomly selected areas of $4 \times 10^3/\mu m^2$ per section.
[c]Within each column, values with the same letter are not significantly different ($P > 0.05$) using Kruskall-Wallis and repeated Mann-Whitney tests.

assessed using a spore germination bioassay. A 5-μl drop of spore suspension (5×10^6/ml) was added to 50-μl drops of extracts on sterile cavity slides. Percentage germination (defined as spores with a germ tube longer than the diameter of the spore) was measured after incubation for 12 h at 25°C. One hundred spores in five replicate drops were observed for each treatment.

RESULTS

Effect of spore concentration on disease expression in petioles of a wilt-susceptible clone

Infusion of petioles from the susceptible clone (S1) with spores resulted in significant browning in the petioles, and extensive mycelial growth occurred within the petiole tissues (Table 1). The severity of the symptoms and the extent of fungal growth were unaffected by the spore concentration (10^2–10^6/ml) used to infuse the petioles (Table 1). In uninoculated petioles little or no browning occurred and little or no fungal growth was observed.

Comparison of wilt-resistant and susceptible clones

In subsequent investigations, 10^5 spores/ml was selected as a standard spore concentration, and the reaction of petioles from known resistant and susceptible clones (R1 and S1) was compared.

Table 2. External symptoms and fungal growth in petiole sections of a resistant and a susceptible clone, 8 days after treatment[a]

Clone	Treatment	Symptoms (browning score)	Fungal growth in petiole[b]
R1	Infused	1·2a[c]	53·2a
	Wet control	0·5a	0·0
	Dry control	0·0	0·0
S1	Infused	2·8b	201·4b
	Wet control	1·0a	0·1
	Dry control	0·2a	0·0

[a]Values are means of 10 replicate palms, one petiole per palm and observations of three sections per petiole for fungal growth.
[b]Total number of hyphae observed in five randomly selected areas of $4 \times 10^3/\mu m^2$ per section.
[c]Within each column, values with the same letter are not significantly different ($P > 0.05$) using Kruskall-Wallis and repeated Mann-Whitney tests.

These clones have been used as standard material in conventional resistance screening trials, in which the degree of infection frequently varied between trials (Mepsted, 1993).

Petioles of R1 remained green, while those of clone S1 were brown and contained significantly more mycelial growth (Table 2). Most conidia in R1 remained ungerminated or were lysed.

At time zero, significantly less spore germination occurred in extracts (dry control and infused) from xylem fluids of R1 than in xylem extracts from S1 (Fig. 1a). Antifungal activity increased in these extracts over time; this increase was most pronounced in extracts from R1 petioles infused with conidia. Little antifungal activity was apparent in xylem fluids from either inoculated or control petioles of clone S1, with *c.* 80–90% spore germination in all treatments (Fig. 1a). Thus both preformed and induced antifungal compound(s) were evident in xylem fluid of R1.

Similarly, antifungal activity accumulated in R1 petiole tissue (Fig. 1b), and by 8 days post-inoculation the extracts from inoculated petioles were completely inhibitory. Preformed antifungal substances were also apparently present in control extracts of R1, as indicated by significantly lower germination rates at time zero compared with extracts from S1 (Fig. 1b). No inhibition of spore germination was observed with extracts from any petioles of S1.

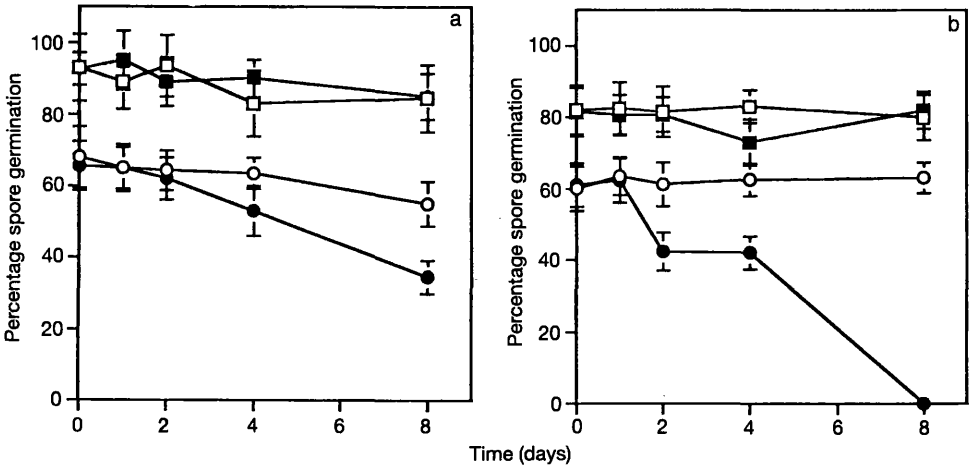

Fig. 1. Accumulation of antifungal compounds, determined by inhibition of spore germination, in (a) petiole xylem fluids and (b) petiole tissue. Xylem fluids were obtained by flushing the petioles with diethyl ether; tissue extracts were obtained by comminuting with sand and diethyl ether. Bars represent standard error. □, susceptible uninoculated; ■, susceptible inoculated; ○, resistant uninoculated; ●, resistant inoculated.

Following the above observations, the experiment was extended by the addition of two more susceptible (S2 and S3) and two more resistant (R2 and R3) clones.

In all cases there was a correlation between wilt resistance and external symptoms, and for five out of six clones resistance was correlated with colonization and the production of antifungal compounds in petioles (Table 3, Fig. 2). Susceptible petioles were easily identified because they were discoloured compared to resistant petioles, which remained green (Fig. 2). Internally, mycelial growth was very limited in petioles from the most resistant clones and extensive in petioles from susceptible clones; petioles from S2 and R1 showed intermediate colonization. Spore germination was significantly lower in extracts from resistant plants than in extracts from

Table 3 External symptoms, fungal colonization and accumulation of antifungal compound(s) in infused petioles of six clones, 8 days after treatment

Clone	Disease resistance/ susceptibility[a]	Symptoms (browning score)	Fungal growth in petiole[b]	Spore germination in tissue extract (%)[c]
R2	0·86[d]	0·9a	2·9a	0
R3	0·94	1·2a	3·7a	15a
R1	1·00	1·7a	19·6ab	25ab
S2	2·02	2·7b	29·7ab	67bc
S3	2·41	2·8b	36·5bc	73c
S1	3·16	2·9b	54·8c	85c

[a]Mean frequency of infection relative to R1 following conventional soil inoculation. In these previous experiments the percentage of inoculated R1 plants with browning of vascular tissue was in the range 22–65%. Values represent the mean of six replicate plants, one petiole per palm and observation of three sections per petiole for fungal growth.
[b]Total number of hyphae observed in five randomly selected areas of $4 \times 10^3/\mu m^2$ per section.
[c]Percentage germination, compared to 86% for water control and 88% for solvent control.
[d]Within each column, values with the same letter are not significantly different ($P > 0.05$) using Kruskall-Wallis and repeated Mann-Whitney tests.

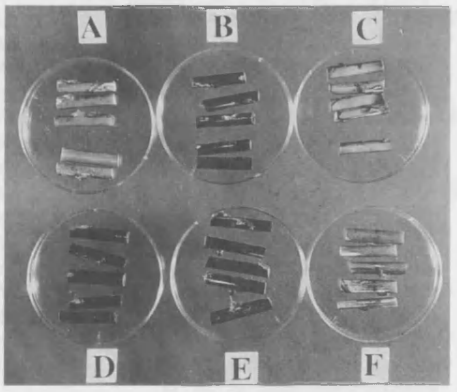

Fig. 2. External symptoms of petioles of six clones following infusion with spores of *Fusarium oxysporum* f. sp. *elaedis*. A, C and F, petioles from resistant clones R1, R2 and R3, respectively; B, D and E, petioles from susceptible clones C1, C2 and C3, respectively.

susceptible plants; complete inhibition of conidia occurred in extracts from R2. Germination in extracts from susceptible plants was not significantly different from that in either water or solvent-only controls (Table 3).

DISCUSSION

Model systems are often required in the study of plant disease, especially for cell-cell interactions of vascular diseases which involve asynchronous chronic invasion of non-living xylem vessels. In some cases, including tomato and *Fusarium oxysporum* f.sp. *lycopersici*, resistance can be expressed in tissue culture (Kroon *et al.*, 1991). The expression of resistance by callus in the oil palm-*Fusarium* interaction could theoretically provide an economical and rapid screen for resistance and allow the study of the underlying resistance mechanisms. However, despite several attempts to study this interaction in tissue culture by manipulation of media, environmental conditions and pathogen inoculum load, resistance was not expressed (Flood & Paul, unpublished data). Several authors have reported that resistance to vascular wilt pathogens is expressed in excised leaves or stems, e.g. with *Verticillium* (Bell, 1967; Tjamos & Smith, 1974; Flood *et al.*, 1978; Flood & Milton, 1982) and with *Fusarium* (Hutson and Smith, 1980; Marley & Hillocks, 1993). The swollen stem base (pseudobulb) of oil palm is difficult to infuse and examine because of its highly complex vascular architecture, and therefore excised oil palm petioles were tested for their ability to express resistance following infusion with conidia.

Infusion of petioles revealed that resistant and susceptible clones could always be distinguished by their external appearance; petioles from resistant clones remained green while those from susceptible clones turned brown. Furthermore, extensive mycelial growth developed in the petioles of the susceptible clones, whereas limited mycelial growth occurred in resistant clones. These observations coincided with the accumulation of antifungal compounds both in xylem fluids and in petiole tissue of a resistant clone; little antifungal activity occurred in susceptible petioles. Furthermore, there was evidence of some preformed antifungal activity in one of the resistant clones. Clones R2 and R3 were always clearly distinguishable from the susceptible clones. However, differences between R1 and S2 and S3 were not always statistically significant. Previous observations of both field and nursery trials have demonstrated that while the frequency of infection of clone R1 is low, if it is infected, severe symptoms will develop; in contrast, clones R2 and R3 are resistant to both infection and subsequent colonization (Mepsted, 1993; Mepsted *et al.*, 1994a, 1994b). Therefore, the failure to distinguish clearly between R1 and some susceptible clones using the petiole infusion technique may reflect the intermediate nature of resistance in this clone.

Antifungal compounds were previously reported from the roots of naturally inoculated oil palm plants (Taquet *et al.*, 1985; Diabate *et al.*, 1990) and from the roots of resistant date palm cultivars inoculated with *F. oxysporum* f.sp. *albedinis* (Assef *et al.*, 1986). Diabate *et al.* (1990) identified antifungal compounds in root tissues as derivatives of benzoic acid and cinnamic acid, but the identity of the compounds detected in this study is not yet known. Current research is attempting to identify the preformed and induced compounds in the petioles of resistant oil palm lines and clones.

This comparatively simple host-pathogen system should now allow a thorough, detailed investigation of potential resistance mechanisms; such as possible differential production of the host hydrolases, chitinases and glucanases (which may explain spore lysis in resistant clones), phenolic biosynthetic enzymes such as phenylalanine ammonia lyase (PAL) (which may contribute to the characteristic browning response) and the elicitation and characterization of induced

antifungal compounds. Furthermore, an investigation of potential pathogenicity factors from *F. oxysporum* f.sp.*elaeidis*, such as toxins or cell-wall-degrading enzymes, can be made in a simplified model system. We already have preliminary experimental evidence for the involvement of fusaric acid and pectin lyase in this host-pathogen interaction (Flood & Cooper, unpublished data).

An effective disease-screening procedure should be simple and rapid, and must clearly differentiate between susceptible and resistant genotypes (Durbin, 1981). Thus, using assessment of external symptoms (browning score), which is the most valuable and rapid of the criteria we employed, this technique should provide a rapid (8 days) alternative to conventional disease resistance screening at the nursery stage, which takes up to 8 months for full development of symptoms (Flood *et al.*, 1989, 1992). A similar approach has been suggested for *Verticillium* wilt of lucerne (Yu *et al.*, 1993).

This technique should also be of considerable value for selection of resistant individual palms in the plantation. Currently, resistant oil palms are selected in the field on the basis of absence of external symptoms. However, these could be 'escapes' or merely tolerant of the disease; low levels of infection can be present in symptomless palms (Mepsted *et al.*, 1991). Thus, breeding from palms selected from progenies with a lower-than-average incidence of wilt symptoms, the current criterion used, does not guarantee the resistance of the *individual* palm. With this novel petiole infusion technique it should be possible to assess individual, high-yielding palms for resistance in the field. Field appraisal of the technique is currently in progress.

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REFERENCES

Aderungboye FC, 1981. Significance of vascular wilt in oil palm plantations in Nigeria. In: Pushparajah E, Poh Soon C, eds. *The Oil Palm in Agriculture in the Eighties, Vol. 2.* Kuala Lumpur: Incorporate Society of Planters.

Anon., 1960. Annual Report of the Cameroon Development Corporation, 1959. In: *Review of Applied Mycology* **40,** 148 (abstract).

Assef GM, Assari K, Vincent EJ, 1986. Occurrence of an antifungal principle in root extract of a Bayoud resistant date palm cultivar. *Netherlands Journal of Plant Pathology* **92,** 43–7.

Bell AA, 1967. Formation of gossypol in infected or chemically irritated tissues of *Gossypium* species. *Phytopathology* **57,** 759–64.

Cooper RM, Wood RKS, 1975. Regulation of synthesis of cell wall degrading enzymes by *Verticillium albo-atrum* and *Fusarium oxysporum* f.sp. *lycopersici. Physiological Plant Pathology* **5,** 135–56.

Diabate S, Taquet B, Renard JL, de Franqueville H, Reiser, P, 1990. HPLC analysis of substances produced by oil palm and implicated in resistance to *Fusarium oxysporum* f.sp. *elaeidis*; prospects for selection. *Oleagineux* **45,** 49–55.

Durbin RD, 1981. Applications. In: Durbin RD, ed. *Toxins in Plant Disease.* New York: Academic Press, 495–565.

Flood J, Milton JM, 1982. Studies on the incompatible/compatible interactions between lucerne leaves and *Verticillium albo-atrum. Physiological Plant Pathology* **21,** 97–104.

Flood J, Khan FZ, Milton JM, 1978. The role of phytoalexins in *Verticillium* wilt of lucerne. *Annals of Applied Biology* **89,** 329–32.

Flood J, Cooper RM, Lees PE, 1989. A comparison of virulence of *Fusarium oxysporum* f.sp *elaeidis* isolated from Malaysia, Brazil and West Africa to clonal oil palm. *Journal of Phytopathology* **124,** 80–8.

Flood J, Mepsted R, Cooper RM, 1992. Potential spread of *Fusarium* wilt of oil palm on contaminated seed and pollen. In: *Proceedings of the Third International Conference on Plant Protection in the Tropics, Kuala Lumpur, March 1990.* Kuala Lumpur: MAPPS, 102–7.

Flood J, Mepsted R, Velez A, Paul T, Cooper RM, 1993. A comparison of virulence of isolates of *Fusarium oxysporum* f.sp. *elaeidis* from Africa and South America. *Plant Pathology* **42,** 168–71.

Hutson IRA, Smith IM, 1980. Phytoalexins and tyloses in tomato cultivars infected with *Fusarium oxysporum* f.sp. *lycopersici* and *Verticillium albo-atrum. Physiological Plant Pathology* **17,** 245–57

Kroon BAH, Scheffer RJ, Elgersma DM, 1991. Interactions between *Fusarium oxysporum* f.sp *lycopersici* and callus of susceptible and resistant tomato lines: fungal growth and phytoalexin accumulation. *Journal of Phytopathology* **132,** 57–64.

Marley P, Hillocks R, 1993. The role of phytoalexins in resistance to *Fusarium* wilt in pigeon pea (*Cajanus cajan*). *Plant Pathology* **42,** 212–8.

Mepsted R, 1993. *Studies on* Fusarium *Wilt of Oil Palm.* Bath: University of Bath, Ph.D.Thesis.

Mepsted R, Nyanduza C, Flood J, Cooper RM, 1991. A non-destructive quantitative method for the assessment of infection of oil palm by *Fusarium oxysporum* f.sp. *elaeidis. Elaeis: The International Journal of Oil Palm Research and Development* **3,** 329–35.

Mepsted R, Flood J, Cooper RM, 1994a. Fusarium

wilt of oil palm; 'susceptibility' of some palms of a 'resistant' clone. *Oleagineux* **49,** 205–8.

Mepsted R, Flood J, Cooper RM, 1994b. Virulence and aggressiveness in *Fusarium oxysporum* f sp. *elaeidis*; implications for screening for disease resistance. *Oleagineux* **49,** 209–12.

Renard, J.L., de Franqueville, H. 1989. Oil palm vascular wilt. *Oleagineux* **44**, 342–7.

Renard JL, Gascon JP, Bachy A, 1972. Research on vascular wilt disease of the oil palm. *Oleagineux* **27,** 581–91.

Taquet B, Ravise A, Renard JL, Kunesch G, 1985. Modulation des reactions de defence du palmier a huile (*Elaeis guineensis)* contre le *Fusarium oxysporum* f.sp.*elaeidis*. *Phytopathologische Zeitschrift* **112,** 298–314.

Tjamos EC, Smith IM, 1974. The role of phytoalexins in the resistance of tomato to *Verticillium* wilt. *Physiological Plant Pathology* **4,** 249–59.

Van de Lande HL, 1984. Vascular wilt disease of oil palm (*Elaeis guineensis)* in Para, Brazil. *Oil Palm News* **28,** 6–10.

Wardlaw CW, 1946. *Fusarium oxysporum* of the oil palm. *Nature* **158,** 71.

Wardlaw CW, 1948. Vascular wilt disease of the oil palm in Nigeria. *Nature* **162,** 850–1.

Yu Kang-Fu, Christie BR, Pauls KP, 1993. A comparison of screening techniques for resistance to *Verticillium* wilt in alfalfa (*Medicago sativa*). *Journal of Phytopathology* **139,** 26–32.

Plant Pathology (1993) **42**, 168–171

Comparison of virulence of isolates of *Fusarium oxysporum* f.sp. *elaeidis* from Africa and South America

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The virulences of three isolates of *Fusarium oxysporum* f.sp. *elaeidis* from West Africa were compared with that of a Brazilian isolate. The Brazilian strain was more virulent and caused disease in all oil-palm lines tested. even those which had been selected for resistance to. and were generally unaffected by, African strains. Differential interactions between hosts and isolates of the pathogen could have serious implications for selection of breeding material and for the extension of oil-palm cultivation in South America.

INTRODUCTION

Intensive monoculture of the oil palm (*Elaeis guineensis*) has resulted in the appearance of many diseases and physiological disorders including vascular wilt, caused by *Fusarium oxysporum* f.sp *elaeidis* (*F.o.* f.sp *elaeidis*). which is the most serious disease of oil palm in West Africa (Turner. 1981). It has been reported from many West African countries including Zaire (Wardlaw. 1946). Cameroon (Anon., 1960) and Ivory Coast (Renard *et al.*. 1972). and widespread losses have resulted (Aderungboye, 1981).

The situation in other oil-palm-growing areas is less clear: there have been unconfirmed reports of the disease from Surinam (Anon., 1951) and Colombia (Sanchez Potes, 1966). and *F. oxysporum* was isolated from diseased oil palms at Denpasa Estate. Para. Brazil in 1983, although its pathogenicity was not established (Van de Lande. 1984). A very limited outbreak also occurred in Ecuador in 1986 (Renard & de Franqueville. 1989).

We established recently that an isolate of *F.o.* f.sp. *elaeidis* from oil palm in Brazil was pathogenic to clonal oil palms (Flood *et al.*, 1989), but the pathogenicity of this isolate to oil-palm lines selected for resistance/tolerance to African isolates was unknown. This aspect forms the basis of the present investigation.

MATERIALS AND METHODS

Plant material

Oil-palm seeds previously treated for 75 days at 39 C to induce germination (Hartley, 1987) were supplied from the Joint Research Scheme (Plantation Lever au Zaire and Société du Culture au Zaire). Binga. Zaire. In total, seven seed crosses were used in the experiments and were chosen to represent a range of resistance/susceptibility from African breeding programmes. Upon arrival. the seeds were soaked for 7 days with daily changes of water and then incubated in plastic bags at 25°C for 3 weeks. Germination began after 7–14 days and usually reached a peak after a further 14 days. Germinated seeds were transferred to plastic seed trays (150 × 210 mm) containing Fisons F_2 compost:Fisons M_2 compost:perlite (1:1:1) and maintained in a heated glasshouse at 22–30°C, 60–90% r.h.. with a 14-h light regime using high-pressure sodium lamps giving 400–700 μmol m^{-2} s^{-1}. At the 1–2-leaf stage, the seedlings were transferred into individual polyethylene bags (80 × 190 mm) containing similar compost and maintained under similar conditions until the 3-leaf stage (about 2 months old) when they were inoculated.

Fungal cultures

Four isolates of *F.o.* f.sp. *elaeidis* were used in these studies. F_3, Y_1 and BOS were from infected oil palms from three plantations in Zaire (isolated by J. Flood and confirmed as *F. oxysporum* by the International Mycological Institute. Kew, UK). Isolate 1379 was from an infected oil palm. Denpasa. Brazil (supplied and identified as *F. oxysporum* by P. E. Nelson, Pennsylvania State University. USA). All four isolates had been previously shown to be pathogenic to susceptible

clonal oil palm (Flood *et al.*, 1989 and unpublished data).

Inoculation

For inoculation, fungal isolates from soil culture were grown on potato dextrose agar (PDA) for 2 days and then five mycelial plugs (8-mm diameter) were added to 100 ml of sucrose-salts medium (Cooper & Wood, 1975). The cultures were maintained for 5 days at 100 r.p.m. and 28°C and then filtered through two layers of sterile lens tissue and muslin to remove mycelial aggregates. The resulting spore suspension was adjusted to $3{\cdot}3 \times 10^7$ spores/ml, and 10 ml of this suspension was applied to the soil at the base of 16 replicate plants. Controls received 10 ml of similarly diluted sucrose–salts medium. The plants were not disturbed nor was the root system deliberately wounded. The inoculated plants were maintained for 26 weeks under similar conditions to those described above. In a preliminary study, seedlings from a wilt-susceptible oil-palm line (cross 1) and from a wilt-resistant line (cross 2) were inoculated with four fungal isolates, while in a more extensive experiment, two isolates were selected: F_3 (from Africa) and 1379 (from Brazil) were tested against five oil-palm lines (crosses A to E) of varying susceptibility and resistance.

Disease assessments

In the initial study, disease progress was assessed by recording plant height every 3 weeks but in the later experiment, several other growth parameters were used. Thus, an increase in height (over the final 6 weeks) was recorded, as were the dry weights of the root systems and of aerial parts. At the end of the experiment all plants were assessed for external symptoms using a wilt index (Flood *et al.*, 1989). For assessment of internal symptoms, plants were split longitudinally to assess vascular discoloration of the 'bulb' tissue (swollen stem base).

RESULTS

All isolates reduced growth of palms of cross 1 (susceptible) by 56–87% of the height of uninoculated palms (Table 1); isolate 1379 (Brazil) induced the greatest stunting, but not significantly so. In contrast, with cross 2 'resistant' the African isolates had no significant effect on plant growth, but the Brazilian isolate caused significant stunting compared to the controls.

Table 1. Growth of oil-palm seedlings following inoculation with isolates of *Fusarium oxysporum* f.sp. *elaeidis*

	Plant height (cm)	
Isolate	Cross 1	Cross 2
BOS	87·3†	105·9*
Y1	65·0†	109·2*
F_3	67·7†	96·4*†
1379	56·4†	72·4†
Control	100·1*	121·2*

Values represent a mean of 16 replicates.
Within each column, figures with the same symbol are not significantly different using ANOVA ($P > 0{\cdot}05$).

To investigate this apparent differential response further, F_3 and 1379 were tested against five other oil-palm lines with varying degrees of susceptibility/resistance. F_3 was chosen as representative of African isolates as, of 10 isolates examined to date, it exhibited a similar degree of virulence to the others (Table 1 and unpublished data). Also, it is used as a standard isolate for inoculation in nursery screening trials for disease resistance in selection programmes in Zaire.

The rates of disease progress and the effects on growth varied depending on the oil-palm lines used, but two patterns were observed (Table 2). Hence, for crosses A and B, all inoculated plants showed widespread chlorosis and necrosis and extensive vascular browning, irrespective of which isolate was used for inoculation; dry weights of aerial parts of all inoculated plants were also significantly reduced. In contrast, plants of crosses C, D and E inoculated with the African isolate were similar to controls with regard to disease symptoms, vascular necrosis and dry weight, but were significantly shorter than controls. The Brazilian isolate caused significantly more severe symptoms on crosses C and D (based on four of the five criteria); similarly with cross E, significantly more vascular necrosis and reduction in dry weight of roots and shoots occurred with this isolate.

DISCUSSION

In oil-palm seedlings, *Fusarium* wilt is characterized by stunting, leaf chlorosis and necrosis and internally by vascular necrosis (Ho *et al.*, 1985; Flood *et al.*, 1989). Using plant height as an assessment of stunting, the Brazilian isolate (1379) was virulent to cross 2, which had been

Table 2. Symptom induction and effects on growth of seedling oil palms inoculated with two isolates of *Fusarium oxysporum* f.sp.*elaeidis*

	Disease[a]		Growth[b]		
	Vascular necrosis (%)	Wilt[c] index	Increase in height (over final 6 weeks) (mm)	Dry weight of roots (g)	Dry weight of shoots (g)
Cross A					
Brazilian isolate	43·4*	2·0*	65·6*	1·9*	8·2*
African isolate	24·7*	1·8*	52·5*	2·3*†	9·2*
Control	0†	0·2†	150·0†	2·8†	11·7†
Cross B					
Brazilian isolate	63·1*	3·4*	39·3*	2·1*	10·0*
African isolate	32·4*†	2·0†	71·9*	2·6*†	11·7*
Control	0†	0‡	137·5†	3·9†	15·9†
Cross C					
Brazilian isolate	35·7*	2·6*	41·8*	1·9*	7·9*
African isolate	3·6*†	1·0†	95·0†	2·3†	11·4†
Control	0†	0·3†	156·9‡	3·0†	13·1†
Cross D					
Brazilian isolate	30·1*	3·4*	70·6*	1·1*	5·9*
African isolate	3·4†	1·2†	103·1†	2·0†	6·9*†
Control	0†	0·3†	141·9‡	2·9†	8·2†
Cross E					
Brazilian isolate	46·0*	3·1*	100·0*	1·9*	7·7*
African isolate	3·5†	1·7*	101·8*	2·2†	9·9†
Control	0†	0·2†	146·6†	3·1†	13·8†

Key: Values represent the mean of sixteen replicate plants.
[a] For each cross within each column, figures with the same symbol are not significantly different using Multiple Comparison Test (Sokal & Rohlf, 1981) ($P > 0{\cdot}05$).
[b] For each cross, within each column, figures with the same symbol are not significantly different using ANOVA ($P > 0{\cdot}05$).
[c] Wilt index based on disease score 0–5 where 0 = no symptoms and 5 = plant death (Flood *et al.* 1989).

bred for resistance in a selection programme in Zaire. In a more extensive study, other parameters were used in conjunction with height measurements. A wilt index and vascular necrosis in the bulbs were used as disease indicators (external and internal symptoms), and dry weights as a basis for assessment of growth. Vascular necrosis is diagnostic for this disease although its absence does not necessarily indicate absence of the pathogen.

Absence of this normally characteristic symptom might be suggestive of tolerance; both resistance and tolerance have been demonstrated in this disease and they are dependent on the fungal isolates and host lines used (Cooper *et al.*, 1989). Quantitative re-isolation from necrotic and from symptomless bulbs, as described by Flood *et al.* (1989), is necessary to distinguish between a resistant and tolerant host-response to infection, but was not practicable in the current study. Here we will only use the term resistance.

Oil-palm lines C, D and E were more resistant to the African isolate of *Fo.* f.sp. *elaeidis* than the other two crosses, based on the development of only slight wilt symptoms. This confirms data from nursery trials where isolate F_3 is used to select resistant material, and also confirms observations made in the field where trees from these

crosses show few symptoms (H. van Amstel, Joint Research Scheme, Binga, Zaire, personal communication, 1989). Nevertheless, significant differences in growth were sometimes found between inoculated and control plants from these crosses. However, a higher inoculum concentration (× 30) was used in this work to challenge the palms than had been used in nursery trials in Zaire, and this may account for the observed differences in growth.

Palm crosses A and B showed wilt symptoms as early as 13 weeks after inoculation with the African isolate (F_3) while even after 26 weeks (end of the experiment) lines C, D and E only exhibited slight symptoms. After 26 weeks, crosses A and B were stunted and had severe necrosis and chlorosis. Cross A was also susceptible in the field but cross B was regarded as having some degree of field tolerance (H. van Amstel, personal communication).

The *F.o.* f.sp. *elaeidis* isolate from Brazil caused disease in all oil-palm lines, even those which showed high degrees of resistance to isolate F_3. These results suggest the existence of different interactions with isolates from different geographical locations as suggested by Stover (1972) for races of *F. oxysporum* f.sp *cubense*, but further experimentation is required before the greater virulence of the Brazilian isolate can be related to the existence of races of *F.o.* f.sp. *elaeidis*.

However, these results do suggest that disease-resistant lines selected from West African breeding programmes could be ineffective against Brazilian isolates. This has serious implications for the extension of the oil-palm industry in South America; isolates from South America should be incorporated in breeding and selection programmes.

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REFERENCES

Aderungboye F.O. (1981) Significance of vascular wilt in oil palm plantations in Nigeria. In: *Oil Palm in the Eighties* Vol. 2 (Ed. by E. Pushparajah & Chew Pol Soon), pp. 1–11. Incorporated Society of Planters, Kuala Lumput, Malaysia.

Anon. (1951) Jaarverslag over 1950. (Annual Report for 1950). 87 pp. Cited in *Review of Applied Mycology* **32,** 546–47.

Anon. (1960) Annual Report of the Cameroons Development Corporation, 1959. 29 pp. Cited in *Review of Applied Mycology* **40,** 148.

Cooper, R.M., Flood, J. & Mepsted, R. (1989) Fusarium wilt of oil palm: transmission, isolate variation, resistance. In: *Vascular Wilt Diseases of Plants* (Ed. E. C. Tjamos & C. H. Beckman) pp. 247–258. Springer-Verlag, Heidelberg & London.

Cooper R.M. & Wood R.K.S. (1975) Regulation of synthesis of cell wall degrading enzymes by *Verticillium albo-atrum* and *F. oxysporum* f.sp *lycopersici*. *Physiological Plant Pathology* **5,** 135–156.

Flood J., Cooper R.M. & Lees P.E. (1989) An investigation of pathogenicity of four isolates of *Fusarium oxysporum* from South America, Africa and Malaysia to clonal oil palm. *Journal of Phytopathology* **124,** 80–88.

Hartley C.W.S. (1987) *The Oil Palm*. Longmans Scientific Press. London, UK.

Ho Y.W., Varghese G. & Taylor G.S. (1985) Pathogenicity of *Fusarium oxysporum* f.sp *elaeidis* from Africa to seedlings of oil palms. *Phytopathogische Zeitschrift* **114,** 193–202.

Renard J.L. Gascon J.P. & Bachy A. (1972) Research on vascular wilt of the oil palm. *Oleagineux* **27,** 581–591.

Renard J.L. & De Franqueville H. (1989) Oil palm vascular wilt. *Oleagineaux* **44,** 341–347.

Sanchez Potes A. (1966) Enfermedades del Algodonero, del Cocotero y de la Palma Africane en Colombia. *Acta Agronica, Palmira* 16 1–13. Cited in *Review of Applied Mycology* **46,** 630.

Stover R.H. (1972) *Banana, Plantain and Abaca Diseases*. Commonwealth Mycological Institute. Kew, London, UK.

Sokal R.R. & Rohlf F.J. (1981) *Biometry. The Principles and Practice of Statistics in Biological Research* (2nd Edn) Freeman and Company. New York, USA.

Turner P.D. (1981) *Oil Palm Diseases and Disorders*. Oxford University Press. Oxford, UK.

Van de Lande H. (1984) Vascular wilt disease of oil palm (*Elaeis guineensis* Jacq.) in Para Brazil. *Oil Palm News* **28,** 6–10.

Wardlaw C.W. (1946) A wilt disease of the oil palm. *Nature (London)* **518,** 712.

Virulence and aggressiveness in *Fusarium oxysporum* f. sp. *elaeidis*; implications for screening for disease resistance.

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Abstract. —The aggressiveness and virulence of 3 isolates of *Fusarium oxysporum* f.sp. *elaeidis* from different regions in West Africa were compared using 14 oil palm clones. Although the resistance ranking of some clones varied with different isolates, overall there was no significant isolate/clone interaction. With isolates from Nigeria and the Ivory Coast there was a good correlation between the frequency of infection and the severity of symptoms in infected plants; no correlation occurred with a Zairean isolate. The significance of these results to selection of disease resistant material is discussed.

Keywords. — Oil palm, *Fusarium oxysporum* f.sp. *elaeidis*, (Foe), resistance screening, virulence, aggressiveness.

INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum* f.sp. *elaeidis* (Foe) is regarded as the most serious disease of oil palm in Africa (Renard, 1976; Turner, 1981).

Although the disease has been controlled at the nursery stage by the application of a fungicide, this is uneconomical on a plantation scale (Renard, 1976) and therefore, research on disease control has concentrated on the development of resistant or tolerant varieties.

Wardlaw , 1946, 1950, observed large differences in the incidence of wilt in adjacent blocks of field palms, and suggested that genetically controlled resistance may exist. He proposed that to save space and time breeding programmes should be expanded to include screening for wilt resistance using seedlings, which were also susceptible to the disease. These ideas formed the basis for the identification of resistant materials in trials in which young plants are artificially inoculated with Foe. Such methods are now used for the assessment of > 600 seedling crosses per annum in the Ivory Coast (de Franqueville and Renard, 1990).

The reliability of the nursery test depends on the use of a pathogen isolate or isolates that are representative of the area where palms are to be planted. In particular, it is important that although isolates may vary in the degree of symptoms they induce (aggressiveness) they do not significantly differ in their ranking for resistance of different palm crosses (virulence) i.e. that races of Foe do not exist. In other *Fusarium* form species affecting tomatoes, (Gerdemann and Finley, 1951), banana (Buddenhagen, 1990) and peas (Goth and Webb,1981), pathogenic races have been demonstrated.

Consequently, several investigations of variability in Foe have been conducted but no differential interactions have been observed. Thus, Prendergast (1957) found no differences in isolates obtained from diseased palms in and around Cowan estate in Nigeria, and although Obuekwe and Osagie (1989) reported differences in symptom development induced by isolates from Nigeria, Zaire and Cameroon there appeared to be no isolate / cross interaction. Furthermore, no difference in virulence was found for isolates obtained from palms exhibiting the acute or chronic forms of wilt in the Ivory Coast (de Franqueville, 1991). The failure to observe differential interactions in nursery trials, led de Franqueville (1991) to state that the selection of resistant material in the Ivory Coast based on inoculation tests with one or a few isolates was valid.

In contrast to these studies, palms bred for resistance in the Cameroon and Ivory Coast have been reported to be much more susceptible in Nigeria than resistant material selected using Nigerian isolates (Aderungboye, 1981; Oritsejafor, 1989). In particular, the material selected as resistant in the Ivory Coast had the highest incidence of wilt (47%) anywhere in Nigeria (Oritsejafor, 1989). Conversely, material selected as resistant against Nigerian isolates has proved susceptible in the Ivory Coast (Renard, 1991, pers. comm.), and material resistant to Zairean isolates was susceptible to an isolate from Brazil (Flood *et al.*, 1993).

It is therefore possible that significant differences in pathogen virulence do occur between different oil palm growing regions. The virulence of isolates can be compared by inoculating standard palm lines in a nursery trial but disease development is strongly affected by environmental conditions and potentially virulent isolates should not be introduced into new areas. Therefore, such experimentation should be performed under standard environmental conditions in an area where oil palm is not grown. Also, to date, all studies of isolate virulence has been conducted on seedling material in which segregation of resistance genes is possible.

Consequently, this paper compares the pathogenicity of 3 isolates of Foe, used in nursery screening trials in Nigeria, Ivory Coast and Zaire, to 14 clones of oil palm, under standardised conditions in the U.K.

MATERIALS AND METHODS

Planting material

Plantlets, from 14 different clones (supplied by Unifield T.C. Ltd, Bedford. U.K.) were initially transferred from test tubes to propagators. The RH was reduced, over 2 months, to about 80% when the palms were transplanted to black polyethylene pots (80 x 190 mm containing 1.2L of compost :- Levingtons

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F2, Levingtons M2, Perlite, in ratio 1:1:1). They were transferred to a glasshouse maintained at 22-30°C and 70-90% RH with a light regime of 500-800 mols m^{-2} s^{-1} Once a month, palms were watered with a liquid fertiliser (Fisons Liquinure, 1 in 45 dilution, containing N, P, K. in the ratio 8:4:4 and trace elements).

Fungal isolates

Three single spore isolates of *F. oxysporum* f. sp. *elaeidis* (identity confirmed by the International Mycological Institute, Bakeham Lane, Egham, Surrey) from Zaire (F3), Ivory Coast (16F) and Nigeria (Abak 1746) were used in these experiments. These isolates are used in screening trials in their respective countries (Renard *et al.*, 1972; Obuekwe and Osagie; 1989; Flood *et al.*, 1993). Stock cultures were stored on sterile soil at 7°C to minimise growth and mutation.

Inoculum production and inoculation

Inocula were produced and plantlets inoculated using techniques described by Flood *et al.*, (1993).

Assessment of disease

Seven months post inoculation plants were assessed for leaf chlorosis and browning of bulb tissue.

Leaf symptoms were assessed with a chlorosis index, with plants being rated from 0 to 5, (0=healthy, 1=slight chlorosis of oldest leaves, 2=significant chlorosis of < 20% of leaves, 3=significant chlorosis of < 40% of leaves, 4=significant chlorosis of 80% of leaves, 5=plant dead.). To assess internal vascular browning, which is synonymous with infection (Prendergast, 1963), the plants were split longitudinally through the swollen stem base ("bulb") and the area of browning in the bulb was assessed using a percentage key (Mepsted, 1992).

TABLE I. — **Comparison of isolate aggressiveness**

Isolate	Frequency of infection (%) #	% browning of bulb(1)	Wilt index(1)
F3	39.7a	55.2b	1.78a
16F	65.3b	64.6c	2.98b
Abak	59.4b	45.6a	1.49a

Values are the mean of 14 clones with 17-24 palms per clone
Browning of bulb tissue indicates infection
(1) Values are the mean of all infected plants
Within each column, values with the same letter are not significantly different (P>0.01, Kruskall Wallis and Mann Whitney U-Test)

RESULTS

Isolates 16F (Ivory Coast) and Abak (Nigeria) were generally equally aggressive in terms of the number of inoculated palms that became infected, and both produced significantly more infected plants than isolate F3 (Zaire) (Table I).

In infected palms, isolate 16F induced significantly more symptoms (both external and internal) than the other two isolates; isolate F3 induced a similar level of leaf symptoms and significantly more internal symptoms than Abak (Table I).

In general, there was very good correlation between isolates for the percentage of inoculated plants that became infected in different clones (correlation coefficient, F3 vs Abak r = 0.849, F3 vs 16F r = 0.772, 16F vs Abak r = 0.768, all significant at P < 0.01).

However, there were some clones where the ranking for 16F is different to that for F3 and Abak (Table II); clone 3 appears to be susceptible to 16F (79% infection) yet relatively resistant to F3 and Abak isolates (35% & 41% respectively), while with clone 7 this effect is (to a lesser extent) reversed. Yet, overall there is no significant clone/isolate interaction (P>0.05, generalised linear regression of a binomial model of frequency of infection, Genstat S release 2.2). Although, if clone 1 were used as standard resistant and any clone with a significantly higher infection rate is defined as susceptible then clones 3 and 7 would vary in their resistance rating depending on the isolate used.

The validity of the 3 disease assessment methods (% infection and % bulb browning or chlorosis index of infected plants) for measuring the resistance of different clones can be compared by correlation analysis (Table III). There was very good correlation between assessment methods for isolates 16F (Fig. lA) and Abak, e.g. clones with a high frequency of infection also had high levels of chlorosis and bulb browning in infected plants. However, for isolate F3 there was no significant correlation between the frequency of infection and degree of bulb browning or leaf chlorosis in diseased palms (Table III, Fig. lB). For example, with F3, clones 3 and UF28 had low frequencies of infection but those plants that did become infected had severe internal symptoms. In part, this lack of correlation may be due to the lower frequency of infection in plants inoculated with F3 and thus values for bulb browning and leaf chlorosis for clone 1 are the mean of only 2 infected plants. However, for all other clones there were or more replicate observations, and if data for clone 1 is removed from the analysis then the correlation between assessment methods is reduced rather than increased, for example, frequency of infection vs % browning of stem tissue, r = -0.018.

DISCUSSION

In terms of the number of inoculated plants that became infected, which is the standard assessment method used in nursery trials, the isolates from Ivory Coast and Nigeria were more aggressive than F3 from Zaire. This may be explained because 16F and Abak have been specifically selected as isolates that produce a high frequency of infection in nursery trials (Renard, pers comm 1991; Obuekwe and Osagie, 1989) while F3 is a representative isolate from Zaire (Flood *et al.*, 1993). In the absence of any isolate/clone interaction, the more aggressive isolates have the advantage of producing symptoms more rapidly and hence shortening screening trials.

However, while these results demonstrate that overall there was no significant isolate / clone interaction, there were a few clones where the ranking varied considerably between isolates. Furthermore, while the Abak isolate can be regarded as more aggressive than F3 (based on frequency of infection), the situation is reversed if the severity of symptoms in infected plants is considered. This result may indicate that the abilities to infect and colonise the host are controlled by different pathogen characteristics. Thus, Abak is able to infect more plants but F3 is more able to colonise any plant invaded and cause more symptoms.

With Abak and 16F isolates, there was very good correlation between % infection and the severity of symptom development across the clones. This indicates that the 3 parameters measured are all good indicators of host resistance or susceptibility. However, with F3 there was no significant correlation between infection rate and symptom development. This suggests that the Zairean isolate F3 (from Binga) may distinguish between 2 different resistance mechanisms. Thus, clone UF28 was resistant to infection but once infected not to vascular colonisation (this effect has been observed several times in this laboratory), while with clone 12 the situation was reversed.

TABLE II. — **A comparison of resistance of 14 clones to 3 isolates of *Fusarium oxysporum***

Isolate					
F3		16F		Abak	
Clone	With: whitout infection	Clone	With: whitout infection	Clone	With: whitout infection
1	2:22a	1	5:19a	1	5:19a
UF28	5:18ab	7	11:12ab	3	9:13ab
2	6:16ab	UF28	12:11ab	UF28	12:12abc
3	8:15abc	6	13:11bc	2	11:10abc
4	7:12abcd	9	13:8bcd	4	12:9bcd
5	8:13bcd	8	14:6bcde	8	13:10bcd
6	10:13bcd	4	12:5bcde	10	12:8bcd
7	10:13bcd	10	13:5bcde	7	14:8bcd
8	9:10bcd	2	15:5bcde	6	15:8bcde
9	10:11bcd	3	19:5bcde	9	15:7bcde
10	10:10bcd	12	16:4bcde	13	15:6bcde
11	9:8bcd	5	18:3cde	5	16:5cde
12	13:7bcd	11	14:1de	12	18:3de
13	14:6d	13	21:1e	11	17:1e

For each isolate, clones are ranked from the most resistant to the susceptible. Within each column, values with the same letter are not significantly different (Chi-squared and Fishers Exact Test $P > 0.05$).
UF 28 is standard resitant clone.

TABLE III. — **Correlation between disease assessment methods for each isolate**

	Correlation coefficient for each isolate		
Assessment correlation	F3	16F	Abak
% infection / % browning	0.207	0.735***	0.669***
% infection / Wilt index	0.333	0.730***	0.576*
% browning / Wilt index	0.746***	0.846***	0.728***

Values were obtained from 14 clones
% infection = % plants with brown bulb tissue
% browning = mean % bulb browning of all infected plants
*$P < 0.05$, *** $P < 0.005$, 12 degrees of freedom

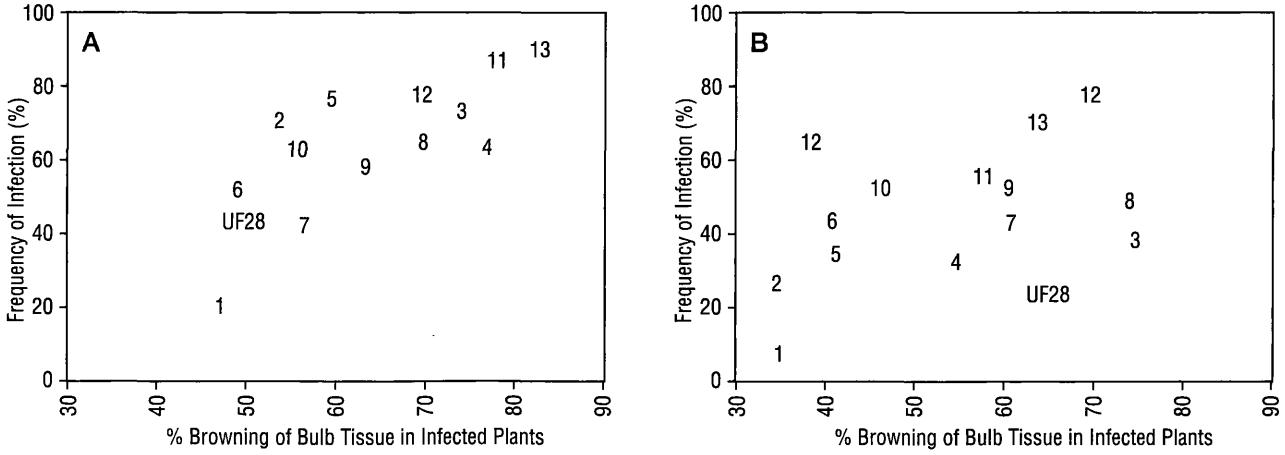

FIG. 1. — Correlation of frequency to severity of infection for 14 clones inoculated with 2 different isolates. Browning of stem tissue is taken to indicate infection.
A = inoculated with 16 F B = inoculated with F3

Two contrasting theories on the inheritance of resistance to Foe have been proposed. Meunier *et al.* (1979) suggested that resistance was inherited through the action of many genes inherited in an additive manner, while de Franqueville and de Greef (1987) proposed that resistance was controlled by the action of just 2 genes. The latter group conducted their experiments at Binga, and therefore their result may reflect the ability of Binga isolates of Foe to distinguish between 2 separate resistance mechanisms.

In summary, the present investigation is in general consistent with the findings of other studies (Meunier *et al.*, 1979; Obuekwe and Osagie, 1989; de Franqueville, 1991) and suggests that while isolates vary in their aggressiveness, their virulence is the same, or at least races are not separable by currently available clones or crosses of oil palm. However, a few clones did not rank in the same manner with different isolates and while this effect was not strong enough to indicate a significant interaction across all clones, it may explain why a few apparently resistant crosses have been susceptible when planted in areas remote from where their resistance was assessed.

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REFERENCES

[1] ADERUNGBOYE F.0.(1981). —Significance of vascular wilt in oil palm plantations in Nigeria. In: *The Oil Palm in Agriculture in the Eighties* (E.PUSHPARAJAH and CHEW POH SOON, eds) Incorporate Society of Planters, Kuala Lumpur: 1-11.

[2] BUDDENHAGEN I.W. (1990). —Banana breeding and *Fusarium* wilt. In:*Fusarium* Wik of Banana (R.C. PLOETZ, ed) *American Phytopathological Society*, St Paul, U.S.A: lO7-114.

[3] FLOOD J, MEPSTED R, VELEZ A., PAUL T. and COOPER R.M.(1993). —Comparison of virulence of isolates of *Fusarium oxysporum* f.sp. *elaeidis* from Africa and South America. *Plant Pathology*, **42**: 168-171.

[4] FRANQUEVILLE H. de and GREEF W. de (1987). —Hereditary transmission of resistance to vascular wilt f the oil palm: Facts and hypotheses. In: *Proceedings of l987 International Oil Palm/ Palm Oil Conference* (A . HALIM HASSAN, CHEW POH SOON, B.J.WOOD and E. PUSHPARAJAH, eds) Palm Oil Research Institute . Kuala Lumpur: 118-129.

[5] FRANQUEVILLE H. de and RENARD J.L. (1990). — Improvement of palm vascular wilt tolerance. Results and development of the disease at the R. Michaux plantation. *Oléagineux*, **45**: 399-405.

[6] FRANQUEVILLE H. de (1991). —Former savannah or former forest: effect of pathogen isolate on the performance of oil palm families with respect to *Fusarium* wilt. *Oléagineux*, **46**: 179-186.

[7] GERDEMANN J.W. and FINLEY A.M. (1951). —The pathogenicity of races 1 and 2 of *Fusarium oxysporum* f.sp. lycopersici. *Phytopathology*, **41**: 238-244.

[8] GOTH R.W. and WEBB R.E. (1981). —Sources and genetics of resistance in vegetable crops. In: *Fungal Wilt Diseases of Plants*. (M.E.MACE, A.A. BELL and C.H. BECKMAN, eds). Academic Press, New York: 377-12.

[9] MEPSTED, R. (1992). —*Fusarium* wilt of oil palm. Ph.D. thesis. University of Bath.

[10] MEUNIER J., RENARD J.L. and QUILLEC G. (1979). —Heredity of resistance to *Fusarium* wilt in the oil palm *Elaeis guineensis* Jacq. *Oléagineux*, **34**: 555-561.

[11] OBUEKWE C.O. and OSAGIE I.J. (1989). —Morphological changes in wiltresistant and wilt-susceptible oil palm progenies and hydrolytic enzyme activities associated with the *Fusarium oxysporum* f.sp. *elaeidis* pathogens. *Oléagineux*, **44**: 393-401.

[12] ORITSEJAFOR J.J. (1989). —Status of the oil palm vascular wilt disease in Nigeria. International Conference on Oil Palm and Palm Products. Benin City: NIFOR.

[13] PRENDERGAST A.G. (1957). —Observations on the epidemiology of vascular wilt disease of the oil palm (*Elaeis guineensis*, Jacq.). *Journal of the West African Institute for Oil Palrn Research*, **2**: 148-175.

[14] PRENDERGAST A.G. (1963). —A method of testing oil palm progress at the nursery stage for resistance to vascular wilt disease caused by *Fusarium oxysporum* Schl. *Journal of the West African Institute for Oil Palm Research*, **4**: 156-175

[15] RENARD J.L., GASCON, J.P. and BACHY A. (1972). —Research on vascular wilt disease of the oil palm. *Oléagineux*, **27**: 581-591.

[16] RENARD J.L. (1976). —Diseases in Africa and South America. In: *Developments in Crop Science* (1), *Oil Palm Research*. (R.H.V. Corley, J.J. Hardon and B.J. Wood, eds). Elsevier Scientific Publishing Company, Amsterdam: 447-466.

[17] RENARD J.L. and FRANQUEVILLE H. de (1989). —Oil palm vascular wilt. *Oléagineux*, **44**, 342-347.

[18] RENARD J.L. and FRANQUEVILLE H. de (1991). —Effectiveness of crop techniques in the integrated control of oil palm vascular wilt. *Oléagineux*, **46**: 255-265.

[19] TURNER P. (1981). —Oil Palm Diseases and Disorders. Oxford: Oxford University Press.

[20] WARDLAW C.W. (1946). —Observations on Diseases of the Oil Palm in the Belgian Congo. Unilever Internal Report

[21] WARDLAW C.W. (1950). —Vascular wilt disease of the oil palm caused by *Fusarium oxysporum* Schl. *Tropical Agriculture*, **27**: 42-47.

RESUME

La virulence et l'agressivité chez *Fusarium oxysporum* f. sp. *elaeidis* : implications pour la sélection de matériel résistant à la maladie

R. MEPSTED, J. FLOOD, T. PAUL, R.M. COOPER, *Oléagineux,* 1994, **49**, N°5, p. 209-212

L'agressivité et la virulence de 3 isolats de *Fusarium oxysporum* f. sp. *elaeidis* provenant de différentes régions d'Afrique occidentale ont été comparées sur 14 clones de palmier à huile. Bien que le niveau de résistance de certains clones varie selon les différents isolats, il n'existe généralement aucune interaction isolat/clone significative. Dans le cas des isolats provenant du Nigéria et de la Côte d'Ivoire, il existe une bonne corrélation entre la fréquence d'infection et l'importance des symptômes chez les plantes infectées; dans le cas de l'isolat provenant du Zaïre il n'y a pas de corrélation. L'intérêt de ces résultats pour la sélection de matériel végétal résistant à la maladie est discuté.

Mots clés. — Palmier à huile, *Fusarium oxysporum* f. sp. *elaeidis* (Foe), sélection de matériel résistant, virulence, agressivité.

RESUMEN

La virulencia y la agresividad en *Fusarium oxysporum* f. sp. *elaeidis*: implicaciones para la selección de material resistente a la enfermedad

R. MEPSTED, J. FLOOD, T. PAUL, R.M. COOPER, *Oléagineux,* 1994, 49, N°5, p. 209-212

Se compararon en 14 clones de palma aceitera la agresividad y la virulencia de 3 cepas de *Fusarium oxysporum* f. sp. *elaeidis* oriundas de diferentes regiones de Africa Occidental. Aunque el nivel de resistencia de algunos clones varia según las diferentes cepas, no existe generalmente ninguna interacción cepa/clón significativa. En el caso de cepas oriundas de Nigeria y de Costa de Marfil, existe una buena correlación entre la frecuencia de infección y la importancia de los síntomas en las plantas infectadas; en el caso de la cepa oriunda del Zaire no hay correlación. Es objeto de discusión el interés de estos resultados para la selección de material vegetal resistente a la enfermedad.

Palabras claves. — Palma aceitera, *Fusarium oxysporum* f. sp. *elaeidis* (Foe), selección de material resistente, virulencia, agresividad.